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# (54) Title: MOLECULES FOR DISEASE DETECTION AND TREATMENT

(57) Abstract: The present invention provides purified disease detection and treatment molecule polynucleotides (mddt). Also encompassed are the polypeptides (MDDT) encoded by mddt. The invention also provides for the use of mddt, or complements, oligonucleotides, or fragments thereof in diagnostic assays. The invention further provides for vectors and host cells containing mddt for the expression of MDDT. The invention additionally provides for the use of isolated and purified MDDT to induce anithodies and to screen libraries of compounds and the use of anti-MDDT antibodies in diagnostic assays. Also provided are microarrays containing mddt and methods of use.



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#### MOLECULES FOR DISEASE DETECTION AND TREATMENT

#### TECHNICAL FIELD

The present invention relates to molecules for disease detection and treatment and to the use of these sequences in the diagnosis, study, prevention, and treatment of diseases associated with, as well as effects of exogenous compounds on, the expression of molecules for disease detection and treatment.

#### **BACKGROUND OF THE INVENTION**

The human genome is comprised of thousands of genes, many encoding gene products that function in the maintenance and growth of the various cells and tissues in the body. Aberrant expression or mutations in these genes and their products is the cause of, or is associated with, a variety of human diseases such as cancer and other cell proliferative disorders. The identification of these genes and their products is the basis of an ever-expanding effort to find markers for early detection of diseases, and targets for their prevention and treatment.

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For example, cancer represents a type of cell proliferative disorder that affects nearly every tissue in the body. A wide variety of molecules, either aberrantly expressed or mutated, can be the cause of, or involved with, various cancers because tissue growth involves complex and ordered patterns of cell proliferation, cell differentiation, and apoptosis. Cell proliferation must be regulated to maintain both the number of cells and their spatial organization. This regulation depends upon the appropriate expression of proteins which control cell cycle progression in response to extracellular signals such as growth factors and other mitogens, and intracellular cues such as DNA damage or nutrient starvation. Molecules which directly or indirectly modulate cell cycle progression fall into several categories, including growth factors and their receptors, second messenger and signal transduction proteins, oncogene products, tumor-suppressor proteins, and mitosis-promoting factors. Aberrant expression or mutations in any of these gene products can result in cell proliferative disorders such as cancer. Oncogenes are genes generally derived from normal genes that, through abnormal expression or mutation, can effect the transformation of a normal cell to a malignant one (oncogenesis). Oncoproteins, encoded by oncogenes, can affect cell proliferation in a variety of ways and include growth factors, growth factor receptors, intracellular signal transducers, nuclear transcription factors, and cell-cycle control proteins. In contrast, tumor-suppressor genes are involved in inhibiting cell proliferation. Mutations which cause reduced or loss of function in tumor-suppressor genes result in aberrant cell proliferation and cancer. Thus a wide variety of genes and their products have been found that are associated with cell proliferative disorders such as cancer, but many more may exist that are yet to be discovered.

DNA-based arrays can provide a simple way to explore the expression of a single polymorphic

gene or a large number of genes. When the expression of a single gene is explored, DNA-based arrays are employed to detect the expression of specific gene variants. For example, a p53 tumor suppressor gene array is used to determine whether individuals are carrying mutations that predispose them to cancer. A cytochrome p450 gene array is useful to determine whether individuals have one of a number of specific mutations that could result in increased drug metabolism, drug resistance or drug toxicity.

DNA-based array technology is especially relevant for the rapid screening of expression of a large number of genes. There is a growing awareness that gene expression is affected in a global fashion. A genetic predisposition, disease or therapeutic treatment may affect, directly or indirectly, the expression of a large number of genes. In some cases the interactions may be expected, such as when the genes are part of the same signaling pathway. In other cases, such as when the genes participate in separate signaling pathways, the interactions may be totally unexpected. Therefore, DNA-based arrays can be used to investigate how genetic predisposition, disease, or therapeutic treatment affects the expression of a large number of genes.

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The discovery of new molecules for disease detection and treatment satisfies a need in the art by providing new compositions which are useful in the diagnosis, study, prevention, and treatment of diseases associated with, as well as effects of exogenous compounds on, the expression of molecules for disease detection and treatment.

#### SUMMARY OF THE INVENTION

The present invention relates to human disease detection and treatment molecule polynucleotides (mddt) as presented in the Sequence Listing. The mddt uniquely identify genes encoding structural, functional, and regulatory disease detection and treatment molecules.

The invention provides an isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-45; b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-45; c) a polynucleotide sequence complementary to a); d) a polynucleotide sequence complementary to b); and e) an RNA equivalent of a) through d). In one alternative, the polynucleotide comprises a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-45. In another alternative, the polynucleotide comprises at least 60 contiguous nucleotides of a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-45; b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-45; c) a polynucleotide sequence complementary to a); d) a

polynucleotide sequence complementary to b); and e) an RNA equivalent of a) through d). The invention further provides a composition for the detection of expression of disease detection and treatment molecule polynucleotides comprising at least one isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-45; b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-45; c) a polynucleotide sequence complementary to a); d) a polynucleotide sequence complementary to b); and e) an RNA equivalent of a) through d); and a detectable label.

The invention also provides a method for detecting a target polynucleotide in a sample, said target polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-45; b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-45; c) a polynucleotide sequence complementary to a); d) a polynucleotide sequence complementary to b); and e) an RNA equivalent of a) through d). The method comprises a) amplifying said target polynucleotide or a fragment thereof using polymerase chain reaction amplification, and b) detecting the presence or absence of said amplified target polynucleotide or fragment thereof, and, optionally, if present, the amount thereof.

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The invention also provides a method for detecting a target polynucleotide in a sample, said target polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-45; b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-45; c) a polynucleotide sequence complementary to a); d) a polynucleotide sequence complementary to b); and e) an RNA equivalent of a) through d). The method comprises a) hybridizing the sample with a probe comprising at least 20 contiguous nucleotides comprising a sequence complementary to said target polynucleotide in the sample, and which probe specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization complex is formed between said probe and said target polynucleotide, and b) detecting the presence or absence of said hybridization complex, and, optionally, if present, the amount thereof. In one alternative, the probe comprises at least 30 contiguous nucleotides. In another alternative, the probe comprises at least 60 contiguous nucleotides.

The invention further provides a recombinant polynucleotide comprising a promoter sequence operably linked to an isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-45; b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a

polynucleotide sequence selected from the group consisting of SEQ ID NO:1-45; c) a polynucleotide sequence complementary to a); d) a polynucleotide sequence complementary to b); and e) an RNA equivalent of a) through d). In one alternative, the invention provides a cell transformed with the recombinant polynucleotide. In another alternative, the invention provides a transgenic organism comprising the recombinant polynucleotide. In a further alternative, the invention provides a method for producing a disease detection and treatment molecule polypeptide, the method comprising a) culturing a cell under conditions suitable for expression of the disease detection and treatment molecule polypeptide, wherein said cell is transformed with the recombinant polynucleotide, and b) recovering the disease detection and treatment molecule polypeptide so expressed.

The invention also provides a purified disease detection and treatment molecule polypeptide (MDDT) encoded by at least one polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-45. Additionally, the invention provides an isolated antibody which specifically binds to the disease detection and treatment molecule polypeptide. The invention further provides a method of identifying a test compound which specifically binds to the disease detection and treatment molecule polypeptide, the method comprising the steps of a) providing a test compound; b) combining the disease detection and treatment molecule polypeptide with the test compound for a sufficient time and under suitable conditions for binding; and c) detecting binding of the disease detection and treatment molecule polypeptide to the test compound, thereby identifying the test compound which specifically binds the disease detection and treatment molecule polypeptide.

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The invention further provides a microarray wherein at least one element of the microarray is an isolated polynucleotide comprising at least 60 contiguous nucleotides of a polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-45; b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-45; c) a polynucleotide sequence complementary to a); d) a polynucleotide sequence complementary to b); and e) an RNA equivalent of a) through d). The invention also provides a method for generating a transcript image of a sample which contains polynucleotides. The method comprises a) labeling the polynucleotides of the sample, b) contacting the elements of the microarray with the labeled polynucleotides of the sample under conditions suitable for the formation of a hybridization complex, and c) quantifying the expression of the polynucleotides in the sample.

Additionally, the invention provides a method for screening a compound for effectiveness in altering expression of a target polynucleotide, wherein said target polynucleotide comprises a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-45; b) a naturally occurring polynucleotide sequence having

at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-45; c) a polynucleotide sequence complementary to a); d) a polynucleotide sequence complementary to b); and e) an RNA equivalent of a) through d). The method comprises a) exposing a sample comprising the target polynucleotide to a compound, and b) detecting altered expression of the target polynucleotide, and c) comparing the expression of the target polynucleotide in the presence of varying amounts of the compound and in the absence of the compound.

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The invention further provides a method for assessing toxicity of a test compound, said method comprising a) treating a biological sample containing nucleic acids with the test compound; b) hybridizing the nucleic acids of the treated biological sample with a probe comprising at least 20 contiguous nucleotides of a polynucleotide comprising a polynucleotide sequence selected from the group consisting of i) a polynucleotide sequence selected from the group consisting of SEO ID NO:1-45; ii) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-45; iii) a polynucleotide sequence complementary to i), iv) a polynucleotide sequence complementary to ii), and v) an RNA equivalent of i)-iv). Hybridization occurs under conditions whereby a specific hybridization complex is formed between said probe and a target polynucleotide in the biological sample, said target polynucleotide comprising a polynucleotide sequence selected from the group consisting of i) a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-45; ii) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-45; iii) a polynucleotide sequence complementary to i), iv) a polynucleotide sequence complementary to ii), and v) an RNA equivalent of i)-iv), and alternatively, the target polynucleotide comprises a fragment of a polynucleotide sequence selected from the group consisting of i)-v) above; c) quantifying the amount of hybridization complex; and d) comparing the amount of hybridization complex in the treated biological sample with the amount of hybridization complex in an untreated biological sample, wherein a difference in the amount of hybridization complex in the treated biological sample is indicative of toxicity of the test compound.

The invention further provides an isolated polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:46-90, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:46-90, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:46-90, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:46-90. In one alternative, the invention provides an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:46-90.

#### **DESCRIPTION OF THE TABLES**

Table 1 shows the sequence identification numbers (SEQ ID NO:s) and template identification numbers (template IDs) corresponding to the polynucleotides of the present invention, along with their GenBank hits (GI Numbers), probability scores, and functional annotations corresponding to the GenBank hits.

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Table 2 shows the sequence identification numbers (SEQ ID NO:s) and template identification numbers (template IDs) corresponding to the polynucleotides of the present invention, along with polynucleotide segments of each template sequence as defined by the indicated "start" and "stop" nucleotide positions. The reading frames of the polynucleotide segments and the Pfam hits, Pfam descriptions, and E-values corresponding to the polypeptide domains encoded by the polynucleotide segments are indicated.

Table 3 shows the sequence identification numbers (SEQ ID NO:s) and template identification numbers (template IDs) corresponding to the polynucleotides of the present invention, along with polynucleotide segments of each template sequence as defined by the indicated "start" and "stop" nucleotide positions. The reading frames of the polynucleotide segments are shown, and the polypeptides encoded by the polynucleotide segments constitute either signal peptide (SP) or transmembrane (TM) domains, as indicated. The membrane topology of the encoded polypeptide sequence is indicated, the N-terminus (N) listed as being oriented to either the cytosolic (in) or non-cytosolic (out) side of the cell membrane or organelle.

Table 4 shows the sequence identification numbers (SEQ ID NO:s) corresponding to the polynucleotides of the present invention, along with component sequence identification numbers (component IDs) corresponding to each template. The component sequences, which were used to assemble the template sequences, are defined by the indicated "start" and "stop" nucleotide positions along each template.

Table 5 shows the tissue distribution profiles for the templates of the invention.

Table 6 shows the sequence identification numbers (SEQ ID NO:s) corresponding to the polypeptides of the present invention, along with the reading frames used to obtain the polypeptide segments, the lengths of the polypeptide segments, the "start" and "stop" nucleotide positions of the polynucleotide sequences used to define the encoded polypeptide segments, the GenBank hits (GI Numbers), probability scores, and functional annotations corresponding to the GenBank hits.

Table 7 summarizes the bioinformatics tools which are useful for analysis of the polynucleotides of the present invention. The first column of Table 7 lists analytical tools, programs, and algorithms, the second column provides brief descriptions thereof, the third column presents

appropriate references, all of which are incorporated by reference herein in their entirety, and the fourth column presents, where applicable, the scores, probability values, and other parameters used to evaluate the strength of a match between two sequences (the higher the score, the greater the homology between two sequences).

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#### DETAILED DESCRIPTION OF THE INVENTION

Before the nucleic acid sequences and methods are presented, it is to be understood that this invention is not limited to the particular machines, methods, and materials described. Although particular embodiments are described, machines, methods, and materials similar or equivalent to these embodiments may be used to practice the invention. The preferred machines, methods, and materials set forth are not intended to limit the scope of the invention which is limited only by the appended claims.

The singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. All technical and scientific terms have the meanings commonly understood by one of ordinary skill in the art. All publications are incorporated by reference for the purpose of describing and disclosing the cell lines, vectors, and methodologies which are presented and which might be used in connection with the invention. Nothing in the specification is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

#### 20 **Definitions**

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As used herein, the lower case "mddt" refers to a nucleic acid sequence, while the upper case "MDDT" refers to an amino acid sequence encoded by mddt. A "full-length" mddt refers to a nucleic acid sequence containing the entire coding region of a gene endogenously expressed in human tissue.

"Adjuvants" are materials such as Freund's adjuvant, mineral gels (aluminum hydroxide), and surface active substances (lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol) which may be administered to increase a host's immunological response.

"Allele" refers to an alternative form of a nucleic acid sequence. Alleles result from a "mutation," a change or an alternative reading of the genetic code. Any given gene may have none, one, or many allelic forms. Mutations which give rise to alleles include deletions, additions, or substitutions of nucleotides. Each of these changes may occur alone, or in combination with the others, one or more times in a given nucleic acid sequence. The present invention encompasses allelic mddt.

"Amino acid sequence" refers to a peptide, a polypeptide, or a protein of either natural or synthetic origin. The amino acid sequence is not limited to the complete, endogenous amino acid

sequence and may be a fragment, epitope, variant, or derivative of a protein expressed by a nucleic acid sequence.

"Amplification" refers to the production of additional copies of a sequence and is carried out using polymerase chain reaction (PCR) technologies well known in the art.

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"Antibody" refers to intact molecules as well as to fragments thereof, such as Fab, F(ab')<sub>2</sub>, and Fv fragments, which are capable of binding the epitopic determinant. Antibodies that bind MDDT polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of interest as the immunizing antigen. The polypeptide or peptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

"Antisense sequence" refers to a sequence capable of specifically hybridizing to a target sequence. The antisense sequence may include DNA, RNA, or any nucleic acid mimic or analog such as peptide nucleic acid (PNA); oligonucleotides having modified backbone linkages such as phosphorothioates, methylphosphonates, or benzylphosphonates; oligonucleotides having modified sugar groups such as 2'-methoxyethyl sugars or 2'-methoxyethoxy sugars; or oligonucleotides having modified bases such as 5-methyl cytosine, 2'-deoxyuracil, or 7-deaza-2'-deoxyguanosine.

"Antisense sequence" refers to a sequence capable of specifically hybridizing to a target sequence. The antisense sequence can be DNA, RNA, or any nucleic acid mimic or analog.

"Antisense technology" refers to any technology which relies on the specific hybridization of an antisense sequence to a target sequence.

A "bin" is a portion of computer memory space used by a computer program for storage of data, and bounded in such a manner that data stored in a bin may be retrieved by the program.

"Biologically active" refers to an amino acid sequence having a structural, regulatory, or biochemical function of a naturally occurring amino acid sequence.

"Clone joining" is a process for combining gene bins based upon the bins' containing sequence information from the same clone. The sequences may assemble into a primary gene transcript as well as one or more splice variants.

"Complementary" describes the relationship between two single-stranded nucleic acid sequences that anneal by base-pairing (5'-A-G-T-3' pairs with its complement 3'-T-C-A-5').

A "component sequence" is a nucleic acid sequence selected by a computer program such as PHRED and used to assemble a consensus or template sequence from one or more component sequences.

A "consensus sequence" or "template sequence" is a nucleic acid sequence which has been assembled from overlapping sequences, using a computer program for fragment assembly such as the GELVIEW fragment assembly system (Genetics Computer Group (GCG), Madison WI) or using a relational database management system (RDMS).

"Conservative amino acid substitutions" are those substitutions that, when made, least interfere with the properties of the original protein, i.e., the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. The table below shows amino acids which may be substituted for an original amino acid in a protein and which are regarded as conservative substitutions.

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	Original Residue	Conservative Substitution
	Ala	Gly, Ser
	Arg	His, Lys
	Asn	Asp, Gln, His
15	Asp	Asn, Glu
	Cys	Ala, Ser
	Gln	Asn, Glu, His
	Glu	Asp, Gln, His
	Gly	Ala
20	His	Asn, Arg, Gln, Glu
	Ile	Leu, Val
	Leu	Ile, Val
	Lys	Arg, Gln, Glu
	Met	Leu, Ile
25	Phe	His, Met, Leu, Trp, Tyr
	Ser	Cys, Thr
	Thr	Ser, Val
	Trp	Phe, Tyr
	Tyr	His, Phe, Trp
30	Val	lle, Leu, Thr

Conservative substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a beta sheet or alpha helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain.

"Deletion" refers to a change in either a nucleic or amino acid sequence in which at least one nucleotide or amino acid residue, respectively, is absent.

"Derivative" refers to the chemical modification of a nucleic acid sequence, such as by replacement of hydrogen by an alkyl, acyl, amino, hydroxyl, or other group.

The terms "element" and "array element" refer to a polynucleotide, polypeptide, or other chemical compound having a unique and defined position on a microarray.

"E-value" refers to the statistical probability that a match between two sequences occurred by chance.

A "fragment" is a unique portion of mddt or MDDT which is identical in sequence to but shorter in length than the parent sequence. A fragment may comprise up to the entire length of the defined sequence, minus one nucleotide/amino acid residue. For example, a fragment may comprise from 10 to 1000 contiguous amino acid residues or nucleotides. A fragment used as a probe, primer, antigen, therapeutic molecule, or for other purposes, may be at least 5, 10, 15, 16, 20, 25, 30, 40, 50, 60, 75, 100, 150, 250 or at least 500 contiguous amino acid residues or nucleotides in length. Fragments may be preferentially selected from certain regions of a molecule. For example, a polypeptide fragment may comprise a certain length of contiguous amino acids selected from the first 250 or 500 amino acids (or first 25% or 50%) of a polypeptide as shown in a certain defined sequence. Clearly these lengths are exemplary, and any length that is supported by the specification, including the Sequence Listing and the figures, may be encompassed by the present embodiments.

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A fragment of mddt comprises a region of unique polynucleotide sequence that specifically identifies mddt, for example, as distinct from any other sequence in the same genome. A fragment of mddt is useful, for example, in hybridization and amplification technologies and in analogous methods that distinguish mddt from related polynucleotide sequences. The precise length of a fragment of mddt and the region of mddt to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

A fragment of MDDT is encoded by a fragment of mddt. A fragment of MDDT comprises a region of unique amino acid sequence that specifically identifies MDDT. For example, a fragment of MDDT is useful as an immunogenic peptide for the development of antibodies that specifically recognize MDDT. The precise length of a fragment of MDDT and the region of MDDT to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

A "full length" nucleotide sequence is one containing at least a start site for translation to a protein sequence, followed by an open reading frame and a stop site, and encoding a "full length" polypeptide.

"Hit" refers to a sequence whose annotation will be used to describe a given template. Criteria for selecting the top hit are as follows: if the template has one or more exact nucleic acid matches, the top hit is the exact match with highest percent identity. If the template has no exact matches but has significant protein hits, the top hit is the protein hit with the lowest E-value. If the template has no significant protein hits, but does have significant non-exact nucleotide hits, the top hit is the nucleotide hit with the lowest E-value.

"Homology" refers to sequence similarity either between a reference nucleic acid sequence and at least a fragment of an mddt or between a reference amino acid sequence and a fragment of an MDDT.

"Hybridization" refers to the process by which a strand of nucleotides anneals with a complementary strand through base pairing. Specific hybridization is an indication that two nucleic acid sequences share a high degree of identity. Specific hybridization complexes form under defined annealing conditions, and remain hybridized after the "washing" step. The defined hybridization conditions include the annealing conditions and the washing step(s), the latter of which is particularly important in determining the stringency of the hybridization process, with more stringent conditions allowing less non-specific binding, i.e., binding between pairs of nucleic acid probes that are not perfectly matched. Permissive conditions for annealing of nucleic acid sequences are routinely determinable and may be consistent among hybridization experiments, whereas wash conditions may be varied among experiments to achieve the desired stringency.

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Generally, stringency of hybridization is expressed with reference to the temperature under which the wash step is carried out. Generally, such wash temperatures are selected to be about 5°C to 20°C lower than the thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength and pH. The  $T_m$  is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. An equation for calculating  $T_m$  and conditions for nucleic acid hybridization is well known and can be found in Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual,  $2^{nd}$  ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; specifically see volume 2, chapter 9.

High stringency conditions for hybridization between polynucleotides of the present invention include wash conditions of  $68^{\circ}$ C in the presence of about  $0.2 \times SSC$  and about 0.1% SDS, for 1 hour. Alternatively, temperatures of about  $65^{\circ}$ C,  $60^{\circ}$ C, or  $55^{\circ}$ C may be used. SSC concentration may be varied from about 0.2 to  $2 \times SSC$ , with SDS being present at about 0.1%. Typically, blocking reagents are used to block non-specific hybridization. Such blocking reagents include, for instance, denatured salmon sperm DNA at about 100- $200 \, \mu g/ml$ . Useful variations on these conditions will be readily apparent to those skilled in the art. Hybridization, particularly under high stringency conditions, may be suggestive of evolutionary similarity between the nucleotides. Such similarity is strongly indicative of a similar role for the nucleotides and their resultant proteins.

Other parameters, such as temperature, salt concentration, and detergent concentration may be varied to achieve the desired stringency. Denaturants, such as formamide at a concentration of about 35-50% v/v, may also be used under particular circumstances, such as RNA:DNA hybridizations. Appropriate hybridization conditions are routinely determinable by one of ordinary skill in the art.

"Immunogenic" describes the potential for a natural, recombinant, or synthetic peptide, epitope, polypeptide, or protein to induce antibody production in appropriate animals, cells, or cell lines.

"Insertion" or "addition" refers to a change in either a nucleic or amino acid sequence in which at least one nucleotide or residue, respectively, is added to the sequence.

"Labeling" refers to the covalent or noncovalent joining of a polynucleotide, polypeptide, or antibody with a reporter molecule capable of producing a detectable or measurable signal.

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"Microarray" is any arrangement of nucleic acids, amino acids, antibodies, etc., on a substrate. The substrate may be a solid support such as beads, glass, paper, nitrocellulose, nylon, or an appropriate membrane.

"Linkers" are short stretches of nucleotide sequence which may be added to a vector or an mddt to create restriction endonuclease sites to facilitate cloning. "Polylinkers" are engineered to incorporate multiple restriction enzyme sites and to provide for the use of enzymes which leave 5' or 3' overhangs (e.g., BamHI, EcoRI, and HindIII) and those which provide blunt ends (e.g., EcoRV, SnaBI, and StuI).

"Naturally occurring" refers to an endogenous polynucleotide or polypeptide that may be isolated from viruses or prokaryotic or eukaryotic cells.

"Nucleic acid sequence" refers to the specific order of nucleotides joined by phosphodiester bonds in a linear, polymeric arrangement. Depending on the number of nucleotides, the nucleic acid sequence can be considered an oligomer, oligonucleotide, or polynucleotide. The nucleic acid can be DNA, RNA, or any nucleic acid analog, such as PNA, may be of genomic or synthetic origin, may be either double-stranded or single-stranded, and can represent either the sense or antisense (complementary) strand.

"Oligomer" refers to a nucleic acid sequence of at least about 6 nucleotides and as many as about 60 nucleotides, preferably about 15 to 40 nucleotides, and most preferably between about 20 and 30 nucleotides, that may be used in hybridization or amplification technologies. Oligomers may be used as, e.g., primers for PCR, and are usually chemically synthesized.

"Operably linked" refers to the situation in which a first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences may be in close proximity or contiguous and, where necessary to join two protein coding regions, in the same reading frame.

"Peptide nucleic acid" (PNA) refers to a DNA mimic in which nucleotide bases are attached to a pseudopeptide backbone to increase stability. PNAs, also designated antigene agents, can prevent gene expression by targeting complementary messenger RNA.

The phrases "percent identity" and "% identity", as applied to polynucleotide sequences, refer to the percentage of residue matches between at least two polynucleotide sequences aligned using a standardized algorithm. Such an algorithm may insert, in a standardized and reproducible way, gaps in the sequences being compared in order to optimize alignment between two sequences, and therefore achieve a more meaningful comparison of the two sequences.

Percent identity between polynucleotide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program. This program is part of the LASERGENE software package, a suite of molecular biological analysis programs (DNASTAR, Madison WI). CLUSTAL V is described in Higgins, D.G. and Sharp, P.M. (1989) CABIOS 5:151-153 and in Higgins, D.G. et al. (1992) CABIOS 8:189-191. For pairwise alignments of polynucleotide sequences, the default parameters are set as follows: Ktuple=2, gap penalty=5, window=4, and "diagonals saved"=4. The "weighted" residue weight table is selected as the default. Percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polynucleotide sequence pairs.

Alternatively, a suite of commonly used and freely available sequence comparison algorithms is provided by the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) (Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410), which is available from several sources, including the NCBI, Bethesda, MD, and on the Internet at http://www.ncbi.nlm.nih.gov/BLAST/. The BLAST software suite includes various sequence analysis programs including "blastn," that is used to determine alignment between a known polynucleotide sequence and other sequences on a variety of databases. Also available is a tool called "BLAST 2 Sequences" that is used for direct pairwise comparison of two nucleotide sequences. "BLAST 2 Sequences" can be accessed and used interactively at http://www.ncbi.nlm.nih.gov/gorf/bl2/. The "BLAST 2 Sequences" tool can be used for both blastn and blastp (discussed below). BLAST programs are commonly used with gap and other parameters set to default settings. For example, to compare two nucleotide sequences, one may use blastn with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such default parameters may be, for example:

Matrix: BLOSUM62

Reward for match: 1

30 Penalty for mismatch: -2

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Open Gap: 5 and Extension Gap: 2 penalties

Gap x drop-off: 50

Expect: 10

Word Size: 11

Filter: on

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Percent identity may be measured over the length of an entire defined sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined sequence, for instance, a fragment of at least 20, at least 30, at least 40, at least 50, at least 70, at least 100, or at least 200 contiguous nucleotides. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in figures or Sequence Listings, may be used to describe a length over which percentage identity may be measured.

Nucleic acid sequences that do not show a high degree of identity may nevertheless encode similar amino acid sequences due to the degeneracy of the genetic code. It is understood that changes in nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid sequences that all encode substantially the same protein.

The phrases "percent identity" and "% identity", as applied to polypeptide sequences, refer to the percentage of residue matches between at least two polypeptide sequences aligned using a standardized algorithm. Methods of polypeptide sequence alignment are well-known. Some alignment methods take into account conservative amino acid substitutions. Such conservative substitutions, explained in more detail above, generally preserve the hydrophobicity and acidity of the substituted residue, thus preserving the structure (and therefore function) of the folded polypeptide.

Percent identity between polypeptide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program (described and referenced above). For pairwise alignments of polypeptide sequences using CLUSTAL V, the default parameters are set as follows: Ktuple=1, gap penalty=3, window=5, and "diagonals saved"=5. The PAM250 matrix is selected as the default residue weight table. As with polynucleotide alignments, the percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polypeptide sequence pairs.

Alternatively the NCBI BLAST software suite may be used. For example, for a pairwise comparison of two polypeptide sequences, one may use the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) with blastp set at default parameters. Such default parameters may be, for example:

Matrix: BLOSUM62

30 Open Gap: 11 and Extension Gap: 1 penalty

Gap x drop-off: 50

Expect: 10
Word Size: 3

Filter: on

Percent identity may be measured over the length of an entire defined polypeptide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polypeptide sequence, for instance, a fragment of at least 15, at least 20, at least 30, at least 40, at least 50, at least 70 or at least 150 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in figures or Sequence Listings, may be used to describe a length over which percentage identity may be measured.

"Post-translational modification" of an MDDT may involve lipidation, glycosylation, phosphorylation, acetylation, racemization, proteolytic cleavage, and other modifications known in the art. These processes may occur synthetically or biochemically. Biochemical modifications will vary by cell type depending on the enzymatic milieu and the MDDT.

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"Probe" refers to mddt or fragments thereof, which are used to detect identical, allelic or related nucleic acid sequences. Probes are isolated oligonucleotides or polynucleotides attached to a detectable label or reporter molecule. Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes. "Primers" are short nucleic acids, usually DNA oligonucleotides, which may be annealed to a target polynucleotide by complementary base-pairing. The primer may then be extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification (and identification) of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR).

Probes and primers as used in the present invention typically comprise at least 15 contiguous nucleotides of a known sequence. In order to enhance specificity, longer probes and primers may also be employed, such as probes and primers that comprise at least 20, 30, 40, 50, 60, 70, 80, 90, 100, or at least 150 consecutive nucleotides of the disclosed nucleic acid sequences. Probes and primers may be considerably longer than these examples, and it is understood that any length supported by the specification, including the figures and Sequence Listing, may be used.

Methods for preparing and using probes and primers are described in the references, for example Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; Ausubel et al., 1987, Current Protocols in Molecular Biology, Greene Publ. Assoc. & Wiley-Intersciences, New York NY; Innis et al., 1990, PCR Protocols, A Guide to Methods and Applications, Academic Press, San Diego CA. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge MA).

Oligonucleotides for use as primers are selected using software known in the art for such purpose. For example, OLIGO 4.06 software is useful for the selection of PCR primer pairs of up to 100 nucleotides each, and for the analysis of oligonucleotides and larger polynucleotides of up to 5,000

nucleotides from an input polynucleotide sequence of up to 32 kilobases. Similar primer selection programs have incorporated additional features for expanded capabilities. For example, the PrimOU primer selection program (available to the public from the Genome Center at University of Texas South West Medical Center, Dallas TX) is capable of choosing specific primers from megabase sequences and is thus useful for designing primers on a genome-wide scope. The Primer3 primer selection program (available to the public from the Whitehead Institute/MIT Center for Genome Research, Cambridge MA) allows the user to input a "mispriming library," in which sequences to avoid as primer binding sites are user-specified. Primer3 is useful, in particular, for the selection of oligonucleotides for microarrays. (The source code for the latter two primer selection programs may also be obtained from their respective sources and modified to meet the user's specific needs.) The PrimeGen program (available to the public from the UK Human Genome Mapping Project Resource Centre, Cambridge UK) designs primers based on multiple sequence alignments, thereby allowing selection of primers that hybridize to either the most conserved or least conserved regions of aligned nucleic acid sequences. Hence, this program is useful for identification of both unique and conserved oligonucleotides and polynucleotide fragments. The oligonucleotides and polynucleotide fragments identified by any of the above selection methods are useful in hybridization technologies, for example, as PCR or sequencing primers, microarray elements, or specific probes to identify fully or partially complementary polynucleotides in a sample of nucleic acids. Methods of oligonucleotide selection are not limited to those described above.

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"Purified" refers to molecules, either polynucleotides or polypeptides that are isolated or separated from their natural environment and are at least 60% free, preferably at least 75% free, and most preferably at least 90% free from other compounds with which they are naturally associated.

A "recombinant nucleic acid" is a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two or more otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques such as those described in Sambrook, <u>supra</u>. The term recombinant includes nucleic acids that have been altered solely by addition, substitution, or deletion of a portion of the nucleic acid. Frequently, a recombinant nucleic acid may include a nucleic acid sequence operably linked to a promoter sequence. Such a recombinant nucleic acid may be part of a vector that is used, for example, to transform a cell.

Alternatively, such recombinant nucleic acids may be part of a viral vector, e.g., based on a vaccinia virus, that could be use to vaccinate a mammal wherein the recombinant nucleic acid is expressed, inducing a protective immunological response in the mammal.

"Regulatory element" refers to a nucleic acid sequence from nontranslated regions of a gene,

and includes enhancers, promoters, introns, and 3' untranslated regions, which interact with host proteins to carry out or regulate transcription or translation.

"Reporter" molecules are chemical or biochemical moieties used for labeling a nucleic acid, an amino acid, or an antibody. They include radionuclides; enzymes; fluorescent, chemiluminescent, or chromogenic agents; substrates; cofactors; inhibitors; magnetic particles; and other moieties known in the art.

An "RNA equivalent," in reference to a DNA sequence, is composed of the same linear sequence of nucleotides as the reference DNA sequence with the exception that all occurrences of the nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose instead of deoxyribose.

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"Sample" is used in its broadest sense. Samples may contain nucleic or amino acids, antibodies, or other materials, and may be derived from any source (e.g., bodily fluids including, but not limited to, saliva, blood, and urine; chromosome(s), organelles, or membranes isolated from a cell; genomic DNA, RNA, or cDNA in solution or bound to a substrate; and cleared cells or tissues or blots or imprints from such cells or tissues).

"Specific binding" or "specifically binding" refers to the interaction between a protein or peptide and its agonist, antibody, antagonist, or other binding partner. The interaction is dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody is specific for epitope "A," the presence of a polypeptide containing epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

"Substitution" refers to the replacement of at least one nucleotide or amino acid by a different nucleotide or amino acid.

"Substrate" refers to any suitable rigid or semi-rigid support including, e.g., membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles or capillaries. The substrate can have a variety of surface forms, such as wells, trenches, pins, channels and pores, to which polynucleotides or polypeptides are bound.

A "transcript image" refers to the collective pattern of gene expression by a particular tissue or cell type under given conditions at a given time.

"Transformation" refers to a process by which exogenous DNA enters a recipient cell.

Transformation may occur under natural or artificial conditions using various methods well known in the art. Transformation may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method is selected based on the host cell being

transformed.

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"Transformants" include stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, as well as cells which transiently express inserted DNA or RNA.

A "transgenic organism," as used herein, is any organism, including but not limited to animals and plants, in which one or more of the cells of the organism contains heterologous nucleic acid introduced by way of human intervention, such as by transgenic techniques well known in the art. The nucleic acid is introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classical cross-breeding, or in vitro fertilization, but rather is directed to the introduction of a recombinant DNA molecule. The transgenic organisms contemplated in accordance with the present invention include bacteria, cyanobacteria, fungi, and plants and animals. The isolated DNA of the present invention can be introduced into the host by methods known in the art, for example infection, transfection, transformation or transconjugation. Techniques for transferring the DNA of the present invention into such organisms are widely known and provided in references such as Sambrook et al. (1989), supra.

A "variant" of a particular nucleic acid sequence is defined as a nucleic acid sequence having at least 25% sequence identity to the particular nucleic acid sequence over a certain length of one of the nucleic acid sequences using blastn with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of nucleic acids may show, for example, at least 30%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% or even at least 98% or greater sequence identity over a certain defined length. The variant may result in "conservative" amino acid changes which do not affect structural and/or chemical properties. A variant may be described as, for example, an "allelic" (as defined above), "splice," "species," or "polymorphic" variant. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternate splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or lack domains that are present in the reference molecule. Species variants are polynucleotide sequences that vary from one species to another. The resulting polypeptides generally will have significant

amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs) in which the polynucleotide sequence varies by one base. The presence of SNPs may be indicative of, for example, a certain population, a disease state, or a propensity for a disease state.

In an alternative, variants of the polynucleotides of the present invention may be generated through recombinant methods. One possible method is a DNA shuffling technique such as MOLECULARBREEDING (Maxygen Inc., Santa Clara CA; described in U.S. Patent Number 5,837,458; Chang, C.-C. et al. (1999) Nat. Biotechnol. 17:793-797; Christians, F.C. et al. (1999) Nat. Biotechnol. 17:259-264; and Crameri, A. et al. (1996) Nat. Biotechnol. 14:315-319) to alter or improve the biological properties of MDDT, such as its biological or enzymatic activity or its ability to bind to other molecules or compounds. DNA shuffling is a process by which a library of gene variants is produced using PCR-mediated recombination of gene fragments. The library is then subjected to selection or screening procedures that identify those gene variants with the desired properties. These preferred variants may then be pooled and further subjected to recursive rounds of DNA shuffling and selection/screening. Thus, genetic diversity is created through "artificial" breeding and rapid molecular evolution. For example, fragments of a single gene containing random point mutations may be recombined, screened, and then reshuffled until the desired properties are optimized. Alternatively, fragments of a given gene may be recombined with fragments of homologous genes in the same gene family, either from the same or different species, thereby maximizing the genetic diversity of multiple naturally occurring genes in a directed and controllable manner.

A "variant" of a particular polypeptide sequence is defined as a polypeptide sequence having at least 40% sequence identity to the particular polypeptide sequence over a certain length of one of the polypeptide sequences using blastp with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of polypeptides may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% or greater sequence identity over a certain defined length of one of the polypeptides.

#### THE INVENTION

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In a particular embodiment, cDNA sequences derived from human tissues and cell lines were aligned based on nucleotide sequence identity and assembled into "consensus" or "template" sequences which are designated by the template identification numbers (template IDs) in column 2 of Table 1. The sequence identification numbers (SEQ ID NO:s) corresponding to the template IDs are shown in column 1. The template sequences have similarity to GenBank sequences, or "hits," as designated by the GI Numbers in column 3. The statistical probability of each GenBank hit is indicated by a probability score in column 4, and the functional annotation corresponding to each GenBank hit is listed in column 5.

The invention incorporates the nucleic acid sequences of these templates as disclosed in the Sequence Listing and the use of these sequences in the diagnosis and treatment of disease states

characterized by defects in disease detection and treatment molecules. The invention further utilizes these sequences in hybridization and amplification technologies, and in particular, in technologies which assess gene expression patterns correlated with specific cells or tissues and their responses <u>in vivo</u> or <u>in vitro</u> to pharmaceutical agents, toxins, and other treatments. In this manner, the sequences of the present invention are used to develop a transcript image for a particular cell or tissue.

# **Derivation of Nucleic Acid Sequences**

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cDNA was isolated from libraries constructed using RNA derived from normal and diseased human tissues and cell lines. The human tissues and cell lines used for cDNA library construction were selected from a broad range of sources to provide a diverse population of cDNAs representative of gene transcription throughout the human body. Descriptions of the human tissues and cell lines used for cDNA library construction are provided in the LIFESEQ database (Incyte Genomics, Inc. (Incyte), Palo Alto CA). Human tissues were broadly selected from, for example, cardiovascular, dermatologic, endocrine, gastrointestinal, hematopoietic/immune system, musculoskeletal, neural, reproductive, and urologic sources.

Cell lines used for cDNA library construction were derived from, for example, leukemic cells, teratocarcinomas, neuroepitheliomas, cervical carcinoma, lung fibroblasts, and endothelial cells. Such cell lines include, for example, THP-1, Jurkat, HUVEC, hNT2, WI38, HeLa, and other cell lines commonly used and available from public depositories (American Type Culture Collection, Manassas VA). Prior to mRNA isolation, cell lines were untreated, treated with a pharmaceutical agent such as 5'-aza-2'-deoxycytidine, treated with an activating agent such as lipopolysaccharide in the case of leukocytic cell lines, or, in the case of endothelial cell lines, subjected to shear stress.

# Sequencing of the cDNAs

Methods for DNA sequencing are well known in the art. Conventional enzymatic methods employ the Klenow fragment of DNA polymerase I, SEQUENASE DNA polymerase (U.S. Biochemical Corporation, Cleveland OH), Taq polymerase (Applied Biosystems, Foster City CA), thermostable T7 polymerase (Amersham Pharmacia Biotech, Inc. (Amersham Pharmacia Biotech), Piscataway NJ), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE amplification system (Life Technologies Inc. (Life Technologies), Gaithersburg MD), to extend the nucleic acid sequence from an oligonucleotide primer annealed to the DNA template of interest. Methods have been developed for the use of both single-stranded and double-stranded templates. Chain termination reaction products may be electrophoresed on urea-polyacrylamide gels and detected either by autoradiography (for radioisotope-labeled nucleotides) or by fluorescence (for

fluorophore-labeled nucleotides). Automated methods for mechanized reaction preparation, sequencing, and analysis using fluorescence detection methods have been developed. Machines used to prepare cDNAs for sequencing can include the MICROLAB 2200 liquid transfer system (Hamilton Company (Hamilton), Reno NV), Peltier thermal cycler (PTC200; MJ Research, Inc. (MJ Research), Watertown MA), and ABI CATALYST 800 thermal cycler (Applied Biosystems). Sequencing can be carried out using, for example, the ABI 373 or 377 (Applied Biosystems) or MEGABACE 1000 (Molecular Dynamics, Inc. (Molecular Dynamics), Sunnyvale CA) DNA sequencing systems, or other automated and manual sequencing systems well known in the art.

The nucleotide sequences of the Sequence Listing have been prepared by current, state-of-the-art, automated methods and, as such, may contain occasional sequencing errors or unidentified nucleotides. Such unidentified nucleotides are designated by an N. These infrequent unidentified bases do not represent a hindrance to practicing the invention for those skilled in the art. Several methods employing standard recombinant techniques may be used to correct errors and complete the missing sequence information. (See, e.g., those described in Ausubel, F.M. et al. (1997) Short Protocols in Molecular Biology, John Wiley & Sons, New York NY; and Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview NY.)

# Assembly of cDNA Sequences

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Human polynucleotide sequences may be assembled using programs or algorithms well known in the art. Sequences to be assembled are related, wholly or in part, and may be derived from a single or many different transcripts. Assembly of the sequences can be performed using such programs as PHRAP (Phils Revised Assembly Program) and the GELVIEW fragment assembly system (GCG), or other methods known in the art.

Alternatively, cDNA sequences are used as "component" sequences that are assembled into "template" or "consensus" sequences as follows. Sequence chromatograms are processed, verified, and quality scores are obtained using PHRED. Raw sequences are edited using an editing pathway known as Block 1 (See, e.g., the LIFESEQ Assembled User Guide, Incyte Genomics, Palo Alto, CA). A series of BLAST comparisons is performed and low-information segments and repetitive elements (e.g., dinucleotide repeats, Alu repeats, etc.) are replaced by "n's", or masked, to prevent spurious matches. Mitochondrial and ribosomal RNA sequences are also removed. The processed sequences are then loaded into a relational database management system (RDMS) which assigns edited sequences to existing templates, if available. When additional sequences are added into the RDMS, a process is initiated which modifies existing templates or creates new templates from works in

progress (i.e., nonfinal assembled sequences) containing queued sequences or the sequences themselves. After the new sequences have been assigned to templates, the templates can be merged into bins. If multiple templates exist in one bin, the bin can be split and the templates reannotated.

Once gene bins have been generated based upon sequence alignments, bins are "clone joined" based upon clone information. Clone joining occurs when the 5' sequence of one clone is present in one bin and the 3' sequence from the same clone is present in a different bin, indicating that the two bins should be merged into a single bin. Only bins which share at least two different clones are merged.

A resultant template sequence may contain either a partial or a full length open reading frame, or all or part of a genetic regulatory element. This variation is due in part to the fact that the full length cDNAs of many genes are several hundred, and sometimes several thousand, bases in length. With current technology, cDNAs comprising the coding regions of large genes cannot be cloned because of vector limitations, incomplete reverse transcription of the mRNA, or incomplete "second strand" synthesis. Template sequences may be extended to include additional contiguous sequences derived from the parent RNA transcript using a variety of methods known to those of skill in the art. Extension may thus be used to achieve the full length coding sequence of a gene.

# Analysis of the cDNA Sequences

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The cDNA sequences are analyzed using a variety of programs and algorithms which are well known in the art. (See, e.g., Ausubel, 1997, <a href="mailto:supra">supra</a>, Chapter 7.7; Meyers, R.A. (Ed.) (1995) <a href="Molecular Biology">Molecular Biology</a> and Biotechnology, Wiley VCH, New York NY, pp. 856-853; and Table 7.) These analyses comprise both reading frame determinations, e.g., based on triplet codon periodicity for particular organisms (Fickett, J.W. (1982) Nucleic Acids Res. 10:5303-5318); analyses of potential start and stop codons; and homology searches.

Computer programs known to those of skill in the art for performing computer-assisted searches for amino acid and nucleic acid sequence similarity, include, for example, Basic Local Alignment Search Tool (BLAST; Altschul, S.F. (1993) J. Mol. Evol. 36:290-300; Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410). BLAST is especially useful in determining exact matches and comparing two sequence fragments of arbitrary but equal lengths, whose alignment is locally maximal and for which the alignment score meets or exceeds a threshold or cutoff score set by the user (Karlin, S. et al. (1988) Proc. Natl. Acad. Sci. USA 85:841-845). Using an appropriate search tool (e.g., BLAST or HMM), GenBank, SwissProt, BLOCKS, PFAM and other databases may be searched for sequences containing regions of homology to a query mddt or MDDT of the present invention.

Other approaches to the identification, assembly, storage, and display of nucleotide and polypeptide sequences are provided in "Relational Database for Storing Biomolecule Information,"

U.S.S.N. 08/947,845, filed October 9, 1997; "Project-Based Full-Length Biomolecular Sequence Database," U.S.S.N. 08/811,758, filed March 6, 1997; and "Relational Database and System for Storing Information Relating to Biomolecular Sequences," U.S.S.N. 09/034,807, filed March 4, 1998, all of which are incorporated by reference herein in their entirety.

Protein hierarchies can be assigned to the putative encoded polypeptide based on, e.g., motif, BLAST, or biological analysis. Methods for assigning these hierarchies are described, for example, in "Database System Employing Protein Function Hierarchies for Viewing Biomolecular Sequence Data," U.S.S.N. 08/812,290, filed March 6, 1997, incorporated herein by reference.

# 10 <u>Human Disease Detection and Treatment Molecule Sequences</u>

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The mddt of the present invention may be used for a variety of diagnostic and therapeutic purposes. For example, an mddt may be used to diagnose a particular condition, disease, or disorder associated with disease detection and treatment molecules. Such conditions, diseases, and disorders include, but are not limited to, a cell proliferative disorder, such as actinic keratosis, arteriosclerosis, 15 atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, a cancer of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, 20 pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; and an autoimmune/inflammatory disorder, such as actinic keratosis, acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome. allergies, ankylosing spondylitis, amyloidosis, anemia, arteriosclerosis, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, bronchitis, bursitis, cholecystitis, cirrhosis, 25 contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, paroxysmal nocturnal hemoglobinuria, hepatitis, hypereosinophilia, irritable bowel syndrome, episodic lymphopenia with lymphocytotoxins, mixed connective tissue disease (MCTD), multiple sclerosis, myasthenia gravis, 30 myocardial or pericardial inflammation, myelofibrosis, osteoarthritis, osteoporosis, pancreatitis, polycythemia vera, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, primary thrombocythemia, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, trauma, and hematopoietic cancer including lymphoma, leukemia, and myeloma. The mddt can be used to detect the presence of, 35

or to quantify the amount of, an mddt-related polynucleotide in a sample. This information is then compared to information obtained from appropriate reference samples, and a diagnosis is established. Alternatively, a polynucleotide complementary to a given mddt can inhibit or inactivate a therapeutically relevant gene related to the mddt.

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# Analysis of mddt Expression Patterns

The expression of mddt may be routinely assessed by hybridization-based methods to determine, for example, the tissue-specificity, disease-specificity, or developmental stage-specificity of mddt expression. For example, the level of expression of mddt may be compared among different cell types or tissues, among diseased and normal cell types or tissues, among cell types or tissues at different developmental stages, or among cell types or tissues undergoing various treatments. This type of analysis is useful, for example, to assess the relative levels of mddt expression in fully or partially differentiated cells or tissues, to determine if changes in mddt expression levels are correlated with the development or progression of specific disease states, and to assess the response of a cell or tissue to a specific therapy, for example, in pharmacological or toxicological studies. Methods for the analysis of mddt expression are based on hybridization and amplification technologies and include membrane-based procedures such as northern blot analysis, high-throughput procedures that utilize, for example, microarrays, and PCR-based procedures.

# 20 Hybridization and Genetic Analysis

The mddt, their fragments, or complementary sequences, may be used to identify the presence of and/or to determine the degree of similarity between two (or more) nucleic acid sequences. The mddt may be hybridized to naturally occurring or recombinant nucleic acid sequences under appropriately selected temperatures and salt concentrations. Hybridization with a probe based on the nucleic acid sequence of at least one of the mddt allows for the detection of nucleic acid sequences, including genomic sequences, which are identical or related to the mddt of the Sequence Listing. Probes may be selected from non-conserved or unique regions of at least one of the polynucleotides of SEQ ID NO:1-45 and tested for their ability to identify or amplify the target nucleic acid sequence using standard protocols.

Polynucleotide sequences that are capable of hybridizing, in particular, to those shown in SEQ ID NO:1-45 and fragments thereof, can be identified using various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) Methods Enzymol. 152:399-407; Kimmel, A.R. (1987) Methods Enzymol. 152:507-511.) Hybridization conditions are discussed in "Definitions."

A probe for use in Southern or northern hybridization may be derived from a fragment of an

mddt sequence, or its complement, that is up to several hundred nucleotides in length and is either single-stranded or double-stranded. Such probes may be hybridized in solution to biological materials such as plasmids, bacterial, yeast, or human artificial chromosomes, cleared or sectioned tissues, or to artificial substrates containing mddt. Microarrays are particularly suitable for identifying the presence of and detecting the level of expression for multiple genes of interest by examining gene expression correlated with, e.g., various stages of development, treatment with a drug or compound, or disease progression. An array analogous to a dot or slot blot may be used to arrange and link polynucleotides to the surface of a substrate using one or more of the following: mechanical (vacuum), chemical, thermal, or UV bonding procedures. Such an array may contain any number of mddt and may be produced by hand or by using available devices, materials, and machines.

Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) Proc. Natl. Acad. Sci. USA 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. USA 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.)

Probes may be labeled by either PCR or enzymatic techniques using a variety of commercially available reporter molecules. For example, commercial kits are available for radioactive and chemiluminescent labeling (Amersham Pharmacia Biotech) and for alkaline phosphatase labeling (Life Technologies). Alternatively, mddt may be cloned into commercially available vectors for the production of RNA probes. Such probes may be transcribed in the presence of at least one labeled nucleotide (e.g., <sup>32</sup>P-ATP, Amersham Pharmacia Biotech).

Additionally the polynucleotides of SEQ ID NO:1-45 or suitable fragments thereof can be used to isolate full length cDNA sequences utilizing hybridization and/or amplification procedures well known in the art, e.g., cDNA library screening, PCR amplification, etc. The molecular cloning of such full length cDNA sequences may employ the method of cDNA library screening with probes using the hybridization, stringency, washing, and probing strategies described above and in Ausubel, <u>supra</u>, Chapters 3, 5, and 6. These procedures may also be employed with genomic libraries to isolate genomic sequences of mddt in order to analyze, e.g., regulatory elements.

#### 30 Genetic Mapping

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Gene identification and mapping are important in the investigation and treatment of almost all conditions, diseases, and disorders. Cancer, cardiovascular disease, Alzheimer's disease, arthritis, diabetes, and mental illnesses are of particular interest. Each of these conditions is more complex than the single gene defects of sickle cell anemia or cystic fibrosis, with select groups of genes being

predictive of predisposition for a particular condition, disease, or disorder. For example, cardiovascular disease may result from malfunctioning receptor molecules that fail to clear cholesterol from the bloodstream, and diabetes may result when a particular individual's immune system is activated by an infection and attacks the insulin-producing cells of the pancreas. In some studies, Alzheimer's disease has been linked to a gene on chromosome 21; other studies predict a different gene and location. Mapping of disease genes is a complex and reiterative process and generally proceeds from genetic linkage analysis to physical mapping.

As a condition is noted among members of a family, a genetic linkage map traces parts of chromosomes that are inherited in the same pattern as the condition. Statistics link the inheritance of particular conditions to particular regions of chromosomes, as defined by RFLP or other markers. (See, for example, Lander, E. S. and Botstein, D. (1986) Proc. Natl. Acad. Sci. USA 83:7353-7357.) Occasionally, genetic markers and their locations are known from previous studies. More often, however, the markers are simply stretches of DNA that differ among individuals. Examples of genetic linkage maps can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) World Wide Web site.

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In another embodiment of the invention, mddt sequences may be used to generate hybridization probes useful in chromosomal mapping of naturally occurring genomic sequences. Either coding or noncoding sequences of mddt may be used, and in some instances, noncoding sequences may be preferable over coding sequences. For example, conservation of an mddt coding sequence among members of a multi-gene family may potentially cause undesired cross hybridization during chromosomal mapping. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355; Price, C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.)

Fluorescent <u>in situ</u> hybridization (FISH) may be correlated with other physical chromosome mapping techniques and genetic map data. (See, e.g., Meyers, <u>supra</u>, pp. 965-968.) Correlation between the location of mddt on a physical chromosomal map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder. The mddt sequences may also be used to detect polymorphisms that are genetically linked to the inheritance of a particular condition, disease, or disorder.

<u>In situ</u> hybridization of chromosomal preparations and genetic mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending existing genetic

maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of the corresponding human chromosome is not known. These new marker sequences can be mapped to human chromosomes and may provide valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once a disease or syndrome has been crudely correlated by genetic linkage with a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) Nature 336:577-580.) The nucleotide sequences of the subject invention may also be used to detect differences in chromosomal architecture due to translocation, inversion, etc., among normal, carrier, or affected individuals.

Once a disease-associated gene is mapped to a chromosomal region, the gene must be cloned in order to identify mutations or other alterations (e.g., translocations or inversions) that may be correlated with disease. This process requires a physical map of the chromosomal region containing the disease-gene of interest along with associated markers. A physical map is necessary for determining the nucleotide sequence of and order of marker genes on a particular chromosomal region. Physical mapping techniques are well known in the art and require the generation of overlapping sets of cloned DNA fragments from a particular organelle, chromosome, or genome. These clones are analyzed to reconstruct and catalog their order. Once the position of a marker is determined, the DNA from that region is obtained by consulting the catalog and selecting clones from that region. The gene of interest is located through positional cloning techniques using hybridization or similar methods.

#### Diagnostic Uses

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The mddt of the present invention may be used to design probes useful in diagnostic assays. Such assays, well known to those skilled in the art, may be used to detect or confirm conditions, disorders, or diseases associated with abnormal levels of mddt expression. Labeled probes developed from mddt sequences are added to a sample under hybridizing conditions of desired stringency. In some instances, mddt, or fragments or oligonucleotides derived from mddt, may be used as primers in amplification steps prior to hybridization. The amount of hybridization complex formed is quantified and compared with standards for that cell or tissue. If mddt expression varies significantly from the standard, the assay indicates the presence of the condition, disorder, or disease. Qualitative or quantitative diagnostic methods may include northern, dot blot, or other membrane or dip-stick based technologies or multiple-sample format technologies such as PCR, enzyme-linked immunosorbent assay (ELISA)-like, pin, or chip-based assays.

The probes described above may also be used to monitor the progress of conditions, disorders, or diseases associated with abnormal levels of mddt expression, or to evaluate the efficacy of a particular therapeutic treatment. The candidate probe may be identified from the mddt that are specific to a given human tissue and have not been observed in GenBank or other genome databases. Such a probe may be used in animal studies, preclinical tests, clinical trials, or in monitoring the treatment of an individual patient. In a typical process, standard expression is established by methods well known in the art for use as a basis of comparison, samples from patients affected by the disorder or disease are combined with the probe to evaluate any deviation from the standard profile, and a therapeutic agent is administered and effects are monitored to generate a treatment profile. Efficacy

10 is evaluated by determining whether the expression progresses toward or returns to the standard normal pattern. Treatment profiles may be generated over a period of several days or several months. Statistical methods well known to those skilled in the art may be use to determine the significance of such therapeutic agents.

The polynucleotides are also useful for identifying individuals from minute biological samples, for example, by matching the RFLP pattern of a sample's DNA to that of an individual's DNA. The polynucleotides of the present invention can also be used to determine the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, an individual can be identified through a unique set of DNA sequences. Once a unique ID database is established for an individual, positive identification of that individual can be made from extremely small tissue samples.

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In a particular aspect, oligonucleotide primers derived from the mddt of the invention may be used to detect single nucleotide polymorphisms (SNPs). SNPs are substitutions, insertions and deletions that are a frequent cause of inherited or acquired genetic disease in humans. Methods of SNP detection include, but are not limited to, single-stranded conformation polymorphism (SSCP) and fluorescent SSCP (fSSCP) methods. In SSCP, oligonucleotide primers derived from mddt are used to amplify DNA using the polymerase chain reaction (PCR). The DNA may be derived, for example, from diseased or normal tissue, biopsy samples, bodily fluids, and the like. SNPs in the DNA cause differences in the secondary and tertiary structures of PCR products in single-stranded form, and these differences are detectable using gel electrophoresis in non-denaturing gels. In fSCCP, the oligonucleotide primers are fluorescently labeled, which allows detection of the amplimers in high-throughput equipment such as DNA sequencing machines. Additionally, sequence database analysis methods, termed in silico SNP (isSNP), are capable of identifying polymorphisms by comparing the sequences of individual overlapping DNA fragments which assemble into a common consensus

sequence. These computer-based methods filter out sequence variations due to laboratory preparation of DNA and sequencing errors using statistical models and automated analyses of DNA sequence chromatograms. In the alternative, SNPs may be detected and characterized by mass spectrometry using, for example, the high throughput MASSARRAY system (Sequenom, Inc., San Diego CA).

DNA-based identification techniques are critical in forensic technology. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, etc., can be amplified using, e.g., PCR, to identify individuals. (See, e.g., Erlich, H. (1992) <a href="PCR Technology">PCR Technology</a>, Freeman and Co., New York, NY). Similarly, polynucleotides of the present invention can be used as polymorphic markers.

There is also a need for reagents capable of identifying the source of a particular tissue. Appropriate reagents can comprise, for example, DNA probes or primers prepared from the sequences of the present invention that are specific for particular tissues. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

The polynucleotides of the present invention can also be used as molecular weight markers on nucleic acid gels or Southern blots, as diagnostic probes for the presence of a specific mRNA in a particular cell type, in the creation of subtracted cDNA libraries which aid in the discovery of novel polynucleotides, in selection and synthesis of oligomers for attachment to an array or other support, and as an antigen to elicit an immune response.

#### 20 <u>Disease Model Systems Using mddt</u>

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The mddt of the invention or their mammalian homologs may be "knocked out" in an animal model system using homologous recombination in embryonic stem (ES) cells. Such techniques are well known in the art and are useful for the generation of animal models of human disease. (See, e.g., U.S. Patent Number 5,175,383 and U.S. Patent Number 5,767,337.) For example, mouse ES cells, such as the mouse 129/SvJ cell line, are derived from the early mouse embryo and grown in culture. The ES cells are transformed with a vector containing the gene of interest disrupted by a marker gene, e.g., the neomycin phosphotransferase gene (neo; Capecchi, M.R. (1989) Science 244:1288-1292). The vector integrates into the corresponding region of the host genome by homologous recombination.

Alternatively, homologous recombination takes place using the Cre-loxP system to knockout a gene of interest in a tissue- or developmental stage-specific manner (Marth, J.D. (1996) Clin. Invest. 97:1999-2002; Wagner, K.U. et al. (1997) Nucleic Acids Res. 25:4323-4330). Transformed ES cells are identified and microinjected into mouse cell blastocysts such as those from the C57BL/6 mouse strain. The blastocysts are surgically transferred to pseudopregnant dams, and the resulting chimeric progeny are genotyped and bred to produce heterozygous or homozygous strains. Transgenic animals thus

generated may be tested with potential therapeutic or toxic agents.

The mddt of the invention may also be manipulated <u>in vitro</u> in ES cells derived from human blastocysts. Human ES cells have the potential to differentiate into at least eight separate cell lineages including endoderm, mesoderm, and ectodermal cell types. These cell lineages differentiate into, for example, neural cells, hematopoietic lineages, and cardiomyocytes (Thomson, J.A. et al. (1998) Science 282:1145-1147).

The mddt of the invention can also be used to create "knockin" humanized animals (pigs) or transgenic animals (mice or rats) to model human disease. With knockin technology, a region of mddt is injected into animal ES cells, and the injected sequence integrates into the animal cell genome.

Transformed cells are injected into blastulae, and the blastulae are implanted as described above.

Transgenic progeny or inbred lines are studied and treated with potential pharmaceutical agents to obtain information on treatment of a human disease. Alternatively, a mammal inbred to overexpress mddt, resulting, e.g., in the secretion of MDDT in its milk, may also serve as a convenient source of that protein (Janne, J. et al. (1998) Biotechnol. Annu. Rev. 4:55-74).

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#### Screening Assays

MDDT encoded by polynucleotides of the present invention may be used to screen for molecules that bind to or are bound by the encoded polypeptides. The binding of the polypeptide and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the polypeptide or the bound molecule. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a ligand or fragment thereof, a natural substrate, or a structural or functional mimetic. (See, Coligan et al., (1991) Current Protocols in Immunology 1(2): Chapter 5.) Similarly, the molecule can be closely related to the natural receptor to which the polypeptide binds, or to at least a fragment of the receptor, e.g., the active site. In either case, the molecule can be rationally designed using known techniques. Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, Drosophila, or E. coli. Cells expressing the polypeptide or cell membrane fractions which contain the expressed polypeptide are then contacted with a test compound and binding, stimulation, or inhibition of activity of either the polypeptide or the molecule is analyzed.

An assay may simply test binding of a candidate compound to the polypeptide, wherein binding is detected by a fluorophore, radioisotope, enzyme conjugate, or other detectable label. Alternatively, the assay may assess binding in the presence of a labeled competitor.

Additionally, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a standard.

Preferably, an ELISA assay using, e.g., a monoclonal or polyclonal antibody, can measure polypeptide level in a sample. The antibody can measure polypeptide level by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.

All of the above assays can be used in a diagnostic or prognostic context. The molecules discovered using these assays can be used to treat disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the polypeptide from suitably manipulated cells or tissues.

#### 15 Transcript Imaging and Toxicological Testing

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Another embodiment relates to the use of mddt to develop a transcript image of a tissue or cell type. A transcript image represents the global pattern of gene expression by a particular tissue or cell type. Global gene expression patterns are analyzed by quantifying the number of expressed genes and their relative abundance under given conditions and at a given time. (See Seilhamer et al.,

"Comparative Gene Transcript Analysis," U.S. Patent Number 5,840,484, expressly incorporated by reference herein.) Thus a transcript image may be generated by hybridizing the polynucleotides of the present invention or their complements to the totality of transcripts or reverse transcripts of a particular tissue or cell type. In one embodiment, the hybridization takes place in high-throughput format, wherein the polynucleotides of the present invention or their complements comprise a subset of a plurality of elements on a microarray. The resultant transcript image would provide a profile of gene activity pertaining to disease detection and treatment molecules.

Transcript images which profile mddt expression may be generated using transcripts isolated from tissues, cell lines, biopsies, or other biological samples. The transcript image may thus reflect mddt expression in vivo, as in the case of a tissue or biopsy sample, or in vitro, as in the case of a cell line.

Transcript images which profile mddt expression may also be used in conjunction with <u>in vitro</u> model systems and preclinical evaluation of pharmaceuticals, as well as toxicological testing of industrial and naturally-occurring environmental compounds. All compounds induce characteristic gene expression patterns, frequently termed molecular fingerprints or toxicant signatures, which are

indicative of mechanisms of action and toxicity (Nuwaysir, E. F. et al. (1999) Mol. Carcinog. 24:153-159; Steiner, S. and Anderson, N. L. (2000) Toxicol. Lett. 112-113:467-71, expressly incorporated by reference herein). If a test compound has a signature similar to that of a compound with known toxicity, it is likely to share those toxic properties. These fingerprints or signatures are most useful and refined when they contain expression information from a large number of genes and gene families. Ideally, a genome-wide measurement of expression provides the highest quality signature. Even genes whose expression is not altered by any tested compounds are important as well, as the levels of expression of these genes are used to normalize the rest of the expression data. The normalization procedure is useful for comparison of expression data after treatment with different compounds. While the assignment of gene function to elements of a toxicant signature aids in interpretation of toxicity mechanisms, knowledge of gene function is not necessary for the statistical matching of signatures which leads to prediction of toxicity. (See, for example, Press Release 00-02 from the National Institute of Environmental Health Sciences, released February 29, 2000, available at http://www.niehs.nih.gov/oc/news/toxchip.htm.) Therefore, it is important and desirable in toxicological screening using toxicant signatures to include all expressed gene sequences.

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In one embodiment, the toxicity of a test compound is assessed by treating a biological sample containing nucleic acids with the test compound. Nucleic acids that are expressed in the treated biological sample are hybridized with one or more probes specific to the polynucleotides of the present invention, so that transcript levels corresponding to the polynucleotides of the present invention may be quantified. The transcript levels in the treated biological sample are compared with levels in an untreated biological sample. Differences in the transcript levels between the two samples are indicative of a toxic response caused by the test compound in the treated sample.

Another particular embodiment relates to the use of MDDT encoded by polynucleotides of the present invention to analyze the proteome of a tissue or cell type. The term proteome refers to the global pattern of protein expression in a particular tissue or cell type. Each protein component of a proteome can be subjected individually to further analysis. Proteome expression patterns, or profiles, are analyzed by quantifying the number of expressed proteins and their relative abundance under given conditions and at a given time. A profile of a cell's proteome may thus be generated by separating and analyzing the polypeptides of a particular tissue or cell type. In one embodiment, the separation is achieved using two-dimensional gel electrophoresis, in which proteins from a sample are separated by isoelectric focusing in the first dimension, and then according to molecular weight by sodium dodecyl sulfate slab gel electrophoresis in the second dimension (Steiner and Anderson, supra). The proteins are visualized in the gel as discrete and uniquely positioned spots, typically by staining the gel with an agent such as Coomassie Blue or silver or fluorescent stains. The optical density of each protein spot is

generally proportional to the level of the protein in the sample. The optical densities of equivalently positioned protein spots from different samples, for example, from biological samples either treated or untreated with a test compound or therapeutic agent, are compared to identify any changes in protein spot density related to the treatment. The proteins in the spots are partially sequenced using, for example, standard methods employing chemical or enzymatic cleavage followed by mass spectrometry. The identity of the protein in a spot may be determined by comparing its partial sequence, preferably of at least 5 contiguous amino acid residues, to the polypeptide sequences of the present invention. In some cases, further sequence data may be obtained for definitive protein identification.

A proteomic profile may also be generated using antibodies specific for MDDT to quantify the levels of MDDT expression. In one embodiment, the antibodies are used as elements on a microarray, and protein expression levels are quantified by exposing the microarray to the sample and detecting the levels of protein bound to each array element (Lueking, A. et al. (1999) Anal. Biochem. 270:103-11; Mendoze, L. G. et al. (1999) Biotechniques 27:778-88). Detection may be performed by a variety of methods known in the art, for example, by reacting the proteins in the sample with a thiol- or aminoreactive fluorescent compound and detecting the amount of fluorescence bound at each array element.

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Toxicant signatures at the proteome level are also useful for toxicological screening, and should be analyzed in parallel with toxicant signatures at the transcript level. There is a poor correlation between transcript and protein abundances for some proteins in some tissues (Anderson, N. L. and Seilhamer, J. (1997) Electrophoresis 18:533-537), so proteome toxicant signatures may be useful in the analysis of compounds which do not significantly affect the transcript image, but which alter the proteomic profile. In addition, the analysis of transcripts in body fluids is difficult, due to rapid degradation of mRNA, so proteomic profiling may be more reliable and informative in such cases.

In another embodiment, the toxicity of a test compound is assessed by treating a biological sample containing proteins with the test compound. Proteins that are expressed in the treated biological sample are separated so that the amount of each protein can be quantified. The amount of each protein is compared to the amount of the corresponding protein in an untreated biological sample. A difference in the amount of protein between the two samples is indicative of a toxic response to the test compound in the treated sample. Individual proteins are identified by sequencing the amino acid residues of the individual proteins and comparing these partial sequences to the MDDT encoded by polynucleotides of the present invention.

In another embodiment, the toxicity of a test compound is assessed by treating a biological sample containing proteins with the test compound. Proteins from the biological sample are incubated with antibodies specific to the MDDT encoded by polynucleotides of the present invention. The amount of protein recognized by the antibodies is quantified. The amount of protein in the treated biological

sample is compared with the amount in an untreated biological sample. A difference in the amount of protein between the two samples is indicative of a toxic response to the test compound in the treated sample.

Transcript images may be used to profile mddt expression in distinct tissue types. This process can be used to determine disease detection and treatment molecule activity in a particular tissue type relative to this activity in a different tissue type. Transcript images may be used to generate a profile of mddt expression characteristic of diseased tissue. Transcript images of tissues before and after treatment may be used for diagnostic purposes, to monitor the progression of disease, and to monitor the efficacy of drug treatments for diseases which affect the activity of disease detection and treatment molecules.

Transcript images of cell lines can be used to assess disease detection and treatment molecule activity and/or to identify cell lines that lack or misregulate this activity. Such cell lines may then be treated with pharmaceutical agents, and a transcript image following treatment may indicate the efficacy of these agents in restoring desired levels of this activity. A similar approach may be used to assess the toxicity of pharmaceutical agents as reflected by undesirable changes in disease detection and treatment molecule activity. Candidate pharmaceutical agents may be evaluated by comparing their associated transcript images with those of pharmaceutical agents of known effectiveness.

# Antisense Molecules

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The polynucleotides of the present invention are useful in antisense technology. Antisense technology or therapy relies on the modulation of expression of a target protein through the specific binding of an antisense sequence to a target sequence encoding the target protein or directing its expression. (See, e.g., Agrawal, S., ed. (1996) Antisense Therapeutics, Humana Press Inc., Totawa NJ; Alama, A. et al. (1997) Pharmacol. Res. 36(3):171-178; Crooke, S.T. (1997) Adv. Phārmacol. 40:1-49; Sharma, H.W. and R. Narayanan (1995) Bioessays 17(12):1055-1063; and Lavrosky, Y. et al. (1997) Biochem. Mol. Med. 62(1):11-22.) An antisense sequence is a polynucleotide sequence capable of specifically hybridizing to at least a portion of the target sequence. Antisense sequences bind to cellular mRNA and/or genomic DNA, affecting translation and/or transcription. Antisense sequences can be DNA, RNA, or nucleic acid mimics and analogs. (See, e.g., Rossi, J.J. et al. (1991) Antisense Res. Dev. 1(3):285-288; Lee, R. et al. (1998) Biochemistry 37(3):900-1010; Pardridge, W.M. et al. (1995) Proc. Natl. Acad. Sci. USA 92(12):5592-5596; and Nielsen, P. E. and Haaima, G. (1997) Chem. Soc. Rev. 96:73-78.) Typically, the binding which results in modulation of expression occurs through hybridization or binding of complementary base pairs. Antisense sequences can also bind to DNA duplexes through specific interactions in the major groove of the double helix.

The polynucleotides of the present invention and fragments thereof can be used as antisense sequences to modify the expression of the polypeptide encoded by mddt. The antisense sequences can be produced <u>ex vivo</u>, such as by using any of the ABI nucleic acid synthesizer series (Applied Biosystems) or other automated systems known in the art. Antisense sequences can also be produced biologically, such as by transforming an appropriate host cell with an expression vector containing the sequence of interest. (See, e.g., Agrawal, <u>supra.</u>)

In therapeutic use, any gene delivery system suitable for introduction of the antisense sequences into appropriate target cells can be used. Antisense sequences can be delivered intracellularly in the form of an expression plasmid which, upon transcription, produces a sequence complementary to at least a portion of the cellular sequence encoding the target protein. (See, e.g., Slater, J.E., et al. (1998) J. Allergy Clin. Immunol. 102(3):469-475; and Scanlon, K.J., et al. (1995) 9(13):1288-1296.) Antisense sequences can also be introduced intracellularly through the use of viral vectors, such as retrovirus and adeno-associated virus vectors. (See, e.g., Miller, A.D. (1990) Blood 76:271; Ausubel, F.M. et al. (1995) Current Protocols in Molecular Biology, John Wiley & Sons, New York NY; Uckert, W. and W. Walther (1994) Pharmacol. Ther. 63(3):323-347.) Other gene delivery mechanisms include liposome-derived systems, artificial viral envelopes, and other systems known in the art. (See, e.g., Rossi, J.J. (1995) Br. Med. Bull. 51(1):217-225; Boado, R.J. et al. (1998) J. Pharm. Sci. 87(11):1308-1315; and Morris, M.C. et al. (1997) Nucleic Acids Res. 25(14):2730-2736.)

#### 20 Expression

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In order to express a biologically active MDDT, the nucleotide sequences encoding MDDT or fragments thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding MDDT and appropriate transcriptional and translational control elements. These methods include <u>in vitro</u> recombinant DNA techniques, synthetic techniques, and <u>in vivo</u> genetic recombination. (See, e.g., Sambrook, <u>supra</u>, Chapters 4, 8, 16, and 17; and Ausubel, <u>supra</u>, Chapters 9, 10, 13, and 16.)

A variety of expression vector/host systems may be utilized to contain and express sequences encoding MDDT. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors (e.g., cauliflower mosaic virus, CaMV, or tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or

animal (mammalian) cell systems. (See, e.g., Sambrook, supra; Ausubel, 1995, supra, Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509; Bitter, G.A. et al. (1987) Methods Enzymol. 153:516-544; Scorer, C.A. et al. (1994) Bio/Technology 12:181-184; Engelhard, E.K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945; Takamatsu, N. (1987) EMBO J. 6:307-311; Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105; The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196; Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. USA 81:3655-3659; and Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355.) Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. (See, e.g., Di Nicola, M. et al. (1998) Cancer Gen. Ther. 5(6):350-356; Yu, M. et al., (1993) Proc. Natl. Acad. Sci. USA 90(13):6340-6344; Buller, R.M. et al. (1985) Nature 317(6040):813-815; McGregor, D.P. et al. (1994) Mol. Immunol. 31(3):219-226; and Verma, I.M. and N. Somia (1997) Nature 389:239-242.) The invention is not limited by the host cell employed.

For long term production of recombinant proteins in mammalian systems, stable expression of MDDT in cell lines is preferred. For example, sequences encoding MDDT can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Any number of selection systems may be used to recover transformed cell lines. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232; Lowy, I. et al. (1980) Cell 22:817-823.; Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. USA 77:3567-3570; Colbere-Garapin, F. et al. (1981) J. Mol. Biol. 150:1-14; Hartman, S.C. and R.C.Mulligan (1988) Proc. Natl. Acad. Sci. USA 85:8047-8051; Rhodes, C.A. (1995) Methods Mol. Biol. 55:121-131.)

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# Therapeutic Uses of mddt

The mddt of the invention may be used for somatic or germline gene therapy. Gene therapy may be performed to (i) correct a genetic deficiency (e.g., in the cases of severe combined immunodeficiency (SCID)-X1 disease characterized by X-linked inheritance (Cavazzana-Calvo, M. et al. (2000) Science 288:669-672), severe combined immunodeficiency syndrome associated with an inherited adenosine deaminase (ADA) deficiency (Blaese, R.M. et al. (1995) Science 270:475-480; Bordignon, C. et al. (1995) Science 270:470-475), cystic fibrosis (Zabner, J. et al. (1993) Cell 75:207-216; Crystal, R.G. et al. (1995) Hum. Gene Therapy 6:643-666; Crystal, R.G. et al. (1995) Hum. Gene Therapy 6:667-703), thalassemias, familial hypercholesterolemia, and hemophilia resulting from Factor

VIII or Factor IX deficiencies (Crystal, R.G. (1995) Science 270:404-410; Verma, I.M. and Somia, N. (1997) Nature 389:239-242)), (ii) express a conditionally lethal gene product (e.g., in the case of cancers which result from unregulated cell proliferation), or (iii) express a protein which affords protection against intracellular parasites (e.g., against human retroviruses, such as human immunodeficiency virus (HIV) (Baltimore, D. (1988) Nature 335:395-396; Poeschla, E. et al. (1996) Proc. Natl. Acad. Sci. USA. 93:11395-11399), hepatitis B or C virus (HBV, HCV); fungal parasites, such as Candida albicans and Paracoccidioides brasiliensis; and protozoan parasites such as Plasmodium falciparum and Trypanosoma cruzi). In the case where a genetic deficiency in mddt expression or regulation causes disease, the expression of mddt from an appropriate population of transduced cells may alleviate the clinical manifestations caused by the genetic deficiency.

In a further embodiment of the invention, diseases or disorders caused by deficiencies in mddt are treated by constructing mammalian expression vectors comprising mddt and introducing these vectors by mechanical means into mddt-deficient cells. Mechanical transfer technologies for use with cells in vivo or ex vitro include (i) direct DNA microinjection into individual cells, (ii) ballistic gold particle delivery, (iii) liposome-mediated transfection, (iv) receptor-mediated gene transfer, and (v) the use of DNA transposons (Morgan, R.A. and Anderson, W.F. (1993) Annu. Rev. Biochem. 62:191-217; Ivics, Z. (1997) Cell 91:501-510; Boulay, J-L. and Récipon, H. (1998) Curr. Opin. Biotechnol. 9:445-450).

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Expression vectors that may be effective for the expression of mddt include, but are not limited to, the PCDNA 3.1, EPITAG, PRCCMV2, PREP, PVAX vectors (Invitrogen, Carlsbad CA), PCMV-SCRIPT, PCMV-TAG, PEGSH/PERV (Stratagene, La Jolla CA), and PTET-OFF, PTET-ON, PTRE2, PTRE2-LUC, PTK-HYG (Clontech, Palo Alto CA). The mddt of the invention may be expressed using (i) a constitutively active promoter, (e.g., from cytomegalovirus (CMV), Rous sarcoma virus (RSV), SV40 virus, thymidine kinase (TK), or β-actin genes), (ii) an inducible promoter (e.g., the tetracycline-regulated promoter (Gossen, M. and Bujard, H. (1992) Proc. Natl. Acad. Sci. U.S.A. 89:5547-5551; Gossen, M. et al., (1995) Science 268:1766-1769; Rossi, F.M.V. and Blau, H.M. (1998) Curr. Opin. Biotechnol. 9:451-456), commercially available in the T-REX plasmid (Invitrogen); the ecdysone-inducible promoter (available in the plasmids PVGRXR and PIND; Invitrogen); the FK506/rapamycin inducible promoter; or the RU486/mifepristone inducible promoter (Rossi, F.M.V. and Blau, H.M. supra), or (iii) a tissue-specific promoter or the native promoter of the endogenous gene encoding MDDT from a normal individual.

Commercially available liposome transformation kits (e.g., the PERFECT LIPID TRANSFECTION KIT, available from Invitrogen) allow one with ordinary skill in the art to deliver polynucleotides to target cells in culture and require minimal effort to optimize experimental

parameters. In the alternative, transformation is performed using the calcium phosphate method (Graham, F.L. and Eb, A.J. (1973) Virology 52:456-467), or by electroporation (Neumann, E. et al. (1982) EMBO J. 1:841-845). The introduction of DNA to primary cells requires modification of these standardized mammalian transfection protocols.

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In another embodiment of the invention, diseases or disorders caused by genetic defects with respect to mddt expression are treated by constructing a retrovirus vector consisting of (i) mddt under the control of an independent promoter or the retrovirus long terminal repeat (LTR) promoter, (ii) appropriate RNA packaging signals, and (iii) a Rev-responsive element (RRE) along with additional retrovirus cis-acting RNA sequences and coding sequences required for efficient vector propagation. Retrovirus vectors (e.g., PFB and PFBNEO) are commercially available (Stratagene) and are based on published data (Riviere, I. et al. (1995) Proc. Natl. Acad. Sci. U.S.A. 92:6733-6737), incorporated by reference herein. The vector is propagated in an appropriate vector producing cell line (VPCL) that expresses an envelope gene with a tropism for receptors on the target cells or a promiscuous envelope protein such as VSVg (Armentano, D. et al. (1987) J. Virol. 61:1647-1650; Bender, M.A. et al. (1987) J. Virol. 61:1639-1646; Adam, M.A. and Miller, A.D. (1988) J. Virol. 62:3802-3806; Dull, T. et al. (1998) J. Virol. 72:8463-8471; Zufferey, R. et al. (1998) J. Virol. 72:9873-9880). U.S. Patent Number 5,910,434 to Rigg ("Method for obtaining retrovirus packaging cell lines producing high transducing efficiency retroviral supernatant") discloses a method for obtaining retrovirus packaging cell lines and is hereby incorporated by reference. Propagation of retrovirus vectors, transduction of a population of cells (e.g., CD4+ T-cells), and the return of transduced cells to a patient are procedures well known to persons skilled in the art of gene therapy and have been well documented (Ranga, U. et al. (1997) J. Virol. 71:7020-7029; Bauer, G. et al. (1997) Blood 89:2259-2267; Bonyhadi, M.L. (1997) J. Virol. 71:4707-4716; Ranga, U. et al. (1998) Proc. Natl. Acad. Sci. U.S.A. 95:1201-1206; Su, L. (1997) Blood 89:2283-2290).

In the alternative, an adenovirus-based gene therapy delivery system is used to deliver mddt to cells which have one or more genetic abnormalities with respect to the expression of mddt. The construction and packaging of adenovirus-based vectors are well known to those with ordinary skill in the art. Replication defective adenovirus vectors have proven to be versatile for importing genes encoding immunoregulatory proteins into intact islets in the pancreas (Csete, M.E. et al. (1995) Transplantation 27:263-268). Potentially useful adenoviral vectors are described in U.S. Patent Number 5,707,618 to Armentano ("Adenovirus vectors for gene therapy"), hereby incorporated by reference. For adenoviral vectors, see also Antinozzi, P.A. et al. (1999) Annu. Rev. Nutr. 19:511-544 and Verma, I.M. and Somia, N. (1997) Nature 18:389:239-242, both incorporated by reference herein.

In another alternative, a herpes-based, gene therapy delivery system is used to deliver mddt to target cells which have one or more genetic abnormalities with respect to the expression of mddt. The use of herpes simplex virus (HSV)-based vectors may be especially valuable for introducing mddt to cells of the central nervous system, for which HSV has a tropism. The construction and packaging of herpes-based vectors are well known to those with ordinary skill in the art. A replication-competent herpes simplex virus (HSV) type 1-based vector has been used to deliver a reporter gene to the eyes of primates (Liu, X. et al. (1999) Exp. Eye Res. 169:385-395). The construction of a HSV-1 virus vector has also been disclosed in detail in U.S. Patent Number 5,804,413 to DeLuca ("Herpes simplex virus strains for gene transfer"), which is hereby incorporated by reference. U.S. Patent Number 5,804,413 teaches the use of recombinant HSV d92 which consists of a genome containing at least one exogenous gene to be transferred to a cell under the control of the appropriate promoter for purposes including human gene therapy. Also taught by this patent are the construction and use of recombinant HSV strains deleted for ICP4, ICP27 and ICP22. For HSV vectors, see also Goins, W. F. et al. 1999 J. Virol. 73:519-532 and Xu, H. et al., (1994) Dev. Biol. 163:152-161, hereby incorporated by reference. The manipulation of cloned herpesvirus sequences, the generation of recombinant virus following the transfection of multiple plasmids containing different segments of the large herpesvirus genomes, the growth and propagation of herpesvirus, and the infection of cells with herpesvirus are techniques well known to those of ordinary skill in the art.

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In another alternative, an alphavirus (positive, single-stranded RNA virus) vector is used to deliver mddt to target cells. The biology of the prototypic alphavirus, Semliki Forest Virus (SFV), has been studied extensively and gene transfer vectors have been based on the SFV genome (Garoff, H. and Li, K-J. (1998) Curr. Opin. Biotech. 9:464-469). During alphavirus RNA replication, a subgenomic RNA is generated that normally encodes the viral capsid proteins. This subgenomic RNA replicates to higher levels than the full-length genomic RNA, resulting in the overproduction of capsid proteins relative to the viral proteins with enzymatic activity (e.g., protease and polymerase). Similarly, inserting mddt into the alphavirus genome in place of the capsid-coding region results in the production of a large number of mddt RNAs and the synthesis of high levels of MDDT in vector transduced cells. While alphavirus infection is typically associated with cell lysis within a few days, the ability to establish a persistent infection in hamster normal kidney cells (BHK-21) with a variant of Sindbis virus (SIN) indicates that the lytic replication of alphaviruses can be altered to suit the needs of the gene therapy application (Dryga, S.A. et al. (1997) Virology 228:74-83). The wide host range of alphaviruses will allow the introduction of mddt into a variety of cell types. The specific transduction of a subset of cells in a population may require the sorting of cells prior to transduction. The methods of manipulating infectious cDNA clones of alphaviruses, performing alphavirus cDNA and RNA

transfections, and performing alphavirus infections, are well known to those with ordinary skill in the art.

#### <u>Antibodies</u>

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Anti-MDDT antibodies may be used to analyze protein expression levels. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, and Fab fragments. For descriptions of and protocols of antibody technologies, see, e.g., Pound J.D. (1998) <u>Immunochemical Protocols</u>, Humana Press, Totowa, NJ.

The amino acid sequence encoded by the mddt of the Sequence Listing may be analyzed by appropriate software (e.g., LASERGENE NAVIGATOR software, DNASTAR) to determine regions of high immunogenicity. The optimal sequences for immunization are selected from the C-terminus, the N-terminus, and those intervening, hydrophilic regions of the polypeptide which are likely to be exposed to the external environment when the polypeptide is in its natural conformation. Analysis used to select appropriate epitopes is also described by Ausubel (1997, <a href="supra">supra</a>, Chapter 11.7). Peptides used for antibody induction do not need to have biological activity; however, they must be antigenic. Peptides used to induce specific antibodies may have an amino acid sequence consisting of at least five amino acids, preferably at least 10 amino acids, and most preferably at least 15 amino acids. A peptide which mimics an antigenic fragment of the natural polypeptide may be fused with another protein such as keyhole hemolimpet cyanin (KLH; Sigma, St. Louis MO) for antibody production. A peptide encompassing an antigenic region may be expressed from an mddt, synthesized as described above, or purified from human cells.

Procedures well known in the art may be used for the production of antibodies. Various hosts including mice, goats, and rabbits, may be immunized by injection with a peptide. Depending on the host species, various adjuvants may be used to increase immunological response.

In one procedure, peptides about 15 residues in length may be synthesized using an ABI 431A peptide synthesizer (Applied Biosystems) using fmoc-chemistry and coupled to KLH (Sigma) by reaction with M-maleimidobenzoyl-N-hydroxysuccinimide ester (Ausubel, 1995, supra). Rabbits are immunized with the peptide-KLH complex in complete Freund's adjuvant. The resulting antisera are tested for antipeptide activity by binding the peptide to plastic, blocking with 1% bovine serum albumin (BSA), reacting with rabbit antisera, washing, and reacting with radioiodinated goat anti-rabbit IgG. Antisera with antipeptide activity are tested for anti-MDDT activity using protocols well known in the art, including ELISA, radioimmunoassay (RIA), and immunoblotting.

In another procedure, isolated and purified peptide may be used to immunize mice (about 100 µg of peptide) or rabbits (about 1 mg of peptide). Subsequently, the peptide is radioiodinated and used

to screen the immunized animals' B-lymphocytes for production of antipeptide antibodies. Positive cells are then used to produce hybridomas using standard techniques. About 20 mg of peptide is sufficient for labeling and screening several thousand clones. Hybridomas of interest are detected by screening with radioiodinated peptide to identify those fusions producing peptide-specific monoclonal antibody. In a typical protocol, wells of a multi-well plate (FAST, Becton-Dickinson, Palo Alto, CA) are coated with affinity-purified, specific rabbit-anti-mouse (or suitable anti-species IgG) antibodies at 10 mg/ml. The coated wells are blocked with 1% BSA and washed and exposed to supernatants from hybridomas. After incubation, the wells are exposed to radiolabeled peptide at 1 mg/ml.

Clones producing antibodies bind a quantity of labeled peptide that is detectable above background. Such clones are expanded and subjected to 2 cycles of cloning. Cloned hybridomas are injected into pristane-treated mice to produce ascites, and monoclonal antibody is purified from the ascitic fluid by affinity chromatography on protein A (Amersham Pharmacia Biotech). Several procedures for the production of monoclonal antibodies, including <u>in vitro</u> production, are described in Pound (<u>supra</u>). Monoclonal antibodies with antipeptide activity are tested for anti-MDDT activity using protocols well known in the art, including ELISA, RIA, and immunoblotting.

Antibody fragments containing specific binding sites for an epitope may also be generated. For example, such fragments include, but are not limited to, the F(ab')2 fragments produced by pepsin digestion of the antibody molecule, and the Fab fragments generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, construction of Fab expression libraries in filamentous bacteriophage allows rapid and easy identification of monoclonal fragments with desired specificity (Pound, supra, Chaps. 45-47). Antibodies generated against polypeptide encoded by mddt can be used to purify and characterize full-length MDDT protein and its activity, binding partners, etc.

#### Assays Using Antibodies

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Anti-MDDT antibodies may be used in assays to quantify the amount of MDDT found in a particular human cell. Such assays include methods utilizing the antibody and a label to detect expression level under normal or disease conditions. The peptides and antibodies of the invention may be used with or without modification or labeled by joining them, either covalently or noncovalently, with a reporter molecule.

Protocols for detecting and measuring protein expression using either polyclonal or monoclonal antibodies are well known in the art. Examples include ELISA, RIA, and fluorescent activated cell sorting (FACS). Such immunoassays typically involve the formation of complexes between the MDDT and its specific antibody and the measurement of such complexes. These and other assays are described in Pound (supra).

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

The disclosures of all patents, applications, and publications mentioned above and below, in particular U.S. Ser. No. 60/185,213, U.S. Ser. No. 60/205,285, U.S. Ser. No. 60/205,232, U.S. Ser. No. 60/205,323, U.S. Ser. No. 60/205,287, U.S. Ser. No. 60/205,324, and U.S. Ser. No. 60/205,286, are hereby expressly incorporated by reference.

10 EXAMPLES

#### I. Construction of cDNA Libraries

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RNA was purchased from CLONTECH Laboratories, Inc. (Palo Alto CA) or isolated from various tissues. Some tissues were homogenized and lysed in guanidinium isothiocyanate, while others were homogenized and lysed in phenol or in a suitable mixture of denaturants, such as TRIZOL (Life Technologies), a monophasic solution of phenol and guanidine isothiocyanate. The resulting lysates were centrifuged over CsCl cushions or extracted with chloroform. RNA was precipitated with either isopropanol or sodium acetate and ethanol, or by other routine methods.

Phenol extraction and precipitation of RNA were repeated as necessary to increase RNA purity. In most cases, RNA was treated with DNase. For most libraries, poly(A+) RNA was isolated using oligo d(T)-coupled paramagnetic particles (Promega Corporation (Promega), Madison WI), OLIGOTEX latex particles (QIAGEN, Inc. (QIAGEN), Valencia CA), or an OLIGOTEX mRNA purification kit (QIAGEN). Alternatively, RNA was isolated directly from tissue lysates using other RNA isolation kits, e.g., the POLY(A)PURE mRNA purification kit (Ambion, Inc., Austin TX).

In some cases, Stratagene was provided with RNA and constructed the corresponding cDNA libraries. Otherwise, cDNA was synthesized and cDNA libraries were constructed with the UNIZAP vector system (Stratagene Cloning Systems, Inc. (Stratagene), La Jolla CA) or SUPERSCRIPT plasmid system (Life Technologies), using the recommended procedures or similar methods known in the art. (See, e.g., Ausubel, 1997, <a href="suppra">suppra</a>, Chapters 5.1 through 6.6.) Reverse transcription was initiated using oligo d(T) or random primers. Synthetic oligonucleotide adapters were ligated to double stranded cDNA, and the cDNA was digested with the appropriate restriction enzyme or enzymes. For most libraries, the cDNA was size-selected (300-1000 bp) using SEPHACRYL S1000, SEPHAROSE CL2B, or SEPHAROSE CL4B column chromatography (Amersham Pharmacia Biotech) or preparative agarose gel electrophoresis. cDNAs were ligated into compatible restriction enzyme sites of the polylinker of a suitable plasmid, e.g., PBLUESCRIPT plasmid (Stratagene), PSPORT1 plasmid

(Life Technologies), PCDNA2.1 plasmid (Invitrogen, Carlsbad CA), PBK-CMV plasmid (Stratagene), or pINCY (Incyte Genomics, Palo Alto CA), or derivatives thereof. Recombinant plasmids were transformed into competent <u>E. coli</u> cells including XL1-Blue, XL1-BlueMRF, or SOLR from Stratagene or DH5α, DH10B, or ElectroMAX DH10B from Life Technologies.

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#### II. Isolation of cDNA Clones

Plasmids were recovered from host cells by <u>in vivo</u> excision using the UNIZAP vector system (Stratagene) or by cell lysis. Plasmids were purified using at least one of the following: the Magic or WIZARD Minipreps DNA purification system (Promega); the AGTC Miniprep purification kit (Edge BioSystems, Gaithersburg MD); and the QIAWELL 8, QIAWELL 8 Plus, and QIAWELL 8 Ultra plasmid purification systems or the R.E.A.L. PREP 96 plasmid purification kit (QIAGEN). Following precipitation, plasmids were resuspended in 0.1 ml of distilled water and stored, with or without lyophilization, at 4°C.

Alternatively, plasmid DNA was amplified from host cell lysates using direct link PCR in a high-throughput format. (Rao, V.B. (1994) Anal. Biochem. 216:1-14.) Host cell lysis and thermal cycling steps were carried out in a single reaction mixture. Samples were processed and stored in 384-well plates, and the concentration of amplified plasmid DNA was quantified fluorometrically using PICOGREEN dye (Molecular Probes, Inc. (Molecular Probes), Eugene OR) and a FLUOROSKAN II fluorescence scanner (Labsystems Oy, Helsinki, Finland).

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#### III. Sequencing and Analysis

cDNA sequencing reactions were processed using standard methods or high-throughput instrumentation such as the ABI CATALYST 800 thermal cycler (Applied Biosystems) or the PTC-200 thermal cycler (MJ Research) in conjunction with the HYDRA microdispenser (Robbins Scientific Corp., Sunnyvale CA) or the MICROLAB 2200 liquid transfer system (Hamilton). cDNA sequencing reactions were prepared using reagents provided by Amersham Pharmacia Biotech or supplied in ABI sequencing kits such as the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Applied Biosystems). Electrophoretic separation of cDNA sequencing reactions and detection of labeled polynucleotides were carried out using the MEGABACE 1000 DNA sequencing system (Molecular Dynamics); the ABI PRISM 373 or 377 sequencing system (Applied Biosystems) in conjunction with standard ABI protocols and base calling software; or other sequence analysis systems known in the art. Reading frames within the cDNA sequences were identified using standard methods (reviewed in Ausubel, 1997, <a href="suppra">suppra</a>, Chapter 7.7). Some of the cDNA sequences were selected for extension using the techniques disclosed in Example VIII.

#### IV. Assembly and Analysis of Sequences

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Component sequences from chromatograms were subject to PHRED analysis and assigned a quality score. The sequences having at least a required quality score were subject to various pre-processing editing pathways to eliminate, e.g., low quality 3' ends, vector and linker sequences, polyA tails, Alu repeats, mitochondrial and ribosomal sequences, bacterial contamination sequences, and sequences smaller than 50 base pairs. In particular, low-information sequences and repetitive elements (e.g., dinucleotide repeats, Alu repeats, etc.) were replaced by "n's", or masked, to prevent spurious matches.

Processed sequences were then subject to assembly procedures in which the sequences were assigned to gene bins (bins). Each sequence could only belong to one bin. Sequences in each gene bin were assembled to produce consensus sequences (templates). Subsequent new sequences were added to existing bins using BLASTn (v.1.4 WashU) and CROSSMATCH. Candidate pairs were identified as all BLAST hits having a quality score greater than or equal to 150. Alignments of at least 82% local identity were accepted into the bin. The component sequences from each bin were assembled using a version of PHRAP. Bins with several overlapping component sequences were assembled using DEEP PHRAP. The orientation (sense or antisense) of each assembled template was determined based on the number and orientation of its component sequences. Template sequences as disclosed in the sequence listing correspond to sense strand sequences (the "forward" reading frames), to the best determination. The complementary (antisense) strands are inherently disclosed herein. The component sequences which were used to assemble each template consensus sequence are listed in Table 4, along with their positions along the template nucleotide sequences.

Bins were compared against each other and those having local similarity of at least 82% were combined and reassembled. Reassembled bins having templates of insufficient overlap (less than 95% local identity) were re-split. Assembled templates were also subject to analysis by STITCHER/EXON MAPPER algorithms which analyze the probabilities of the presence of splice variants, alternatively spliced exons, splice junctions, differential expression of alternative spliced genes across tissue types or disease states, etc. These resulting bins were subject to several rounds of the above assembly procedures.

Once gene bins were generated based upon sequence alignments, bins were clone joined based upon clone information. If the 5' sequence of one clone was present in one bin and the 3' sequence from the same clone was present in a different bin, it was likely that the two bins actually belonged together in a single bin. The resulting combined bins underwent assembly procedures to regenerate the consensus sequences.

The final assembled templates were subsequently annotated using the following procedure. Template sequences were analyzed using BLASTn (v2.0, NCBI) versus gbpri (GenBank version 120). "Hits" were defined as an exact match having from 95% local identity over 200 base pairs through 100% local identity over 100 base pairs, or a homolog match having an E-value, i.e. a probability score, of  $\leq 1 \times 10^{-8}$ . The hits were subject to frameshift FASTx versus GENPEPT (GenBank version 120). (See Table 7). In this analysis, a homolog match was defined as having an E-value of  $\leq 1 \times 10^{-8}$ . The assembly method used above was described in "System and Methods for Analyzing Biomolecular Sequences," U.S.S.N. 09/276,534, filed March 25, 1999, and the LIFESEQ Gold user manual (Incyte) both incorporated by reference herein.

Following assembly, template sequences were subjected to motif, BLAST, and functional analyses, and categorized in protein hierarchies using methods described in, e.g., "Database System Employing Protein Function Hierarchies for Viewing Biomolecular Sequence Data," U.S.S.N. 08/812,290, filed March 6, 1997; "Relational Database for Storing Biomolecular Information," U.S.S.N. 08/947,845, filed October 9, 1997; "Project-Based Full-Length Biomolecular Sequence Database," U.S.S.N. 08/811,758, filed March 6, 1997; and "Relational Database and System for Storing Information Relating to Biomolecular Sequences," U.S.S.N. 09/034,807, filed March 4, 1998, all of which are incorporated by reference herein.

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The template sequences were further analyzed by translating each template in all three forward reading frames and searching each translation against the Pfam database of hidden Markov model-based protein families and domains using the HMMER software package (available to the public from Washington University School of Medicine, St. Louis MO). Regions of templates which, when translated, contain similarity to Pfam consensus sequences are reported in Table 2, along with descriptions of Pfam protein domains and families. Only those Pfam hits with an E-value of  $\leq 1 \times 10^{-3}$  are reported. (See also World Wide Web site http://pfam.wustl.edu/ for detailed descriptions of Pfam protein domains and families.)

Additionally, the template sequences were translated in all three forward reading frames, and each translation was searched against hidden Markov models for signal peptides using the HMMER software package. Construction of hidden Markov models and their usage in sequence analysis has been described. (See, for example, Eddy, S.R. (1996) Curr. Opin. Str. Biol. 6:361-365.) Only those signal peptide hits with a cutoff score of 11 bits or greater are reported. A cutoff score of 11 bits or greater corresponds to at least about 91-94% true-positives in signal peptide prediction. Template sequences were also translated in all three forward reading frames, and each translation was searched against TMAP, a program that uses weight matrices to delineate transmembrane segments on protein sequences and determine orientation, with respect to the cell cytosol (Persson, B. and P. Argos (1994) J.

Mol. Biol. 237:182-192; Persson, B. and P. Argos (1996) Protein Sci. 5:363-371.) Regions of templates which, when translated, contain similarity to signal peptide or transmembrane consensus sequences are reported in Table 3.

The results of HMMER analysis as reported in Tables 2 and 3 may support the results of BLAST analysis as reported in Table 1 or may suggest alternative or additional properties of template-encoded polypeptides not previously uncovered by BLAST or other analyses.

Template sequences are further analyzed using the bioinformatics tools listed in Table 7, or using sequence analysis software known in the art such as MACDNASIS PRO software (Hitachi Software Engineering, South San Francisco CA) and LASERGENE software (DNASTAR). Template sequences may be further queried against public databases such as the GenBank rodent, mammalian, vertebrate, prokaryote, and eukaryote databases.

The template sequences were translated to derive the corresponding longest open reading frame as presented by the polypeptide sequences. Alternatively, a polypeptide of the invention may begin at any of the methionine residues within the full length translated polypeptide. Polypeptide sequences were subsequently analyzed by querying against the GenBank protein database (GENPEPT, (GenBank version 121)). Full length polynucleotide sequences are also analyzed using MACDNASIS PRO software (Hitachi Software Engineering, South San Francisco CA) and LASERGENE software (DNASTAR). Polynucleotide and polypeptide sequence alignments are generated using default parameters specified by the CLUSTAL algorithm as incorporated into the MEGALIGN multisequence alignment program (DNASTAR), which also calculates the percent identity between aligned sequences.

Table 6 shows sequences with homology to the polypeptides of the invention as identified by BLAST analysis against the GenBank protein (GENPEPT) database. Column 1 shows the polypeptide sequence identification number (SEQ ID NO:) for the polypeptide segments of the invention. Column 2 shows the reading frame used in the translation of the polynucleotide sequences encoding the polypeptide segments. Column 3 shows the length of the translated polypeptide segments. Columns 4 and 5 show the start and stop nucleotide positions of the polynucleotide sequences encoding the polypeptide segments. Column 6 shows the GenBank identification number (GI Number) of the nearest GenBank homolog. Column 7 shows the probability score for the match between each polypeptide and its GenBank homolog. Column 8 shows the annotation of the GenBank homolog.

# V. Analysis of Polynucleotide Expression

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Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, supra, ch. 7; Ausubel, 1995, supra, ch. 4 and 16.)

Analogous computer techniques applying BLAST were used to search for identical or related molecules in cDNA databases such as GenBank or LIFESEQ (Incyte Genomics). This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or similar.

5 The basis of the search is the product score, which is defined as:

BLAST Score x	Percent Identity
5 x minimum {length(S	Seq. 1), length(Seq. 2)}

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. The product score is a normalized value between 0 and 100, and is calculated as follows: the BLAST score is multiplied by the percent nucleotide identity and the product is divided by (5 times the length of the shorter of the two sequences). The BLAST score is calculated by assigning a score of +5 for every base that matches in a high-scoring segment pair (HSP), and -4 for every mismatch. Two sequences may share more than one HSP (separated by gaps). If there is more than one HSP, then the pair with the highest BLAST score is used to calculate the product score. The product score represents a balance between fractional overlap and quality in a BLAST alignment. For example, a product score of 100 is produced only for 100% identity over the entire length of the shorter of the two sequences being compared. A product score of 70 is produced either by 100% identity and 70% overlap at one end, or by 88% identity and 100% overlap at the other. A product score of 50 is produced either by 100% identity and 50% overlap at one end, or 79% identity and 100% overlap.

# VI. Tissue Distribution Profiling

A tissue distribution profile is determined for each template by compiling the cDNA library
tissue classifications of its component cDNA sequences. Each component sequence, is derived from a
cDNA library constructed from a human tissue. Each human tissue is classified into one of the
following categories: cardiovascular system; connective tissue; digestive system; embryonic structures;
endocrine system; exocrine glands; genitalia, female; genitalia, male; germ cells; hemic and immune
system; liver; musculoskeletal system; nervous system; pancreas; respiratory system; sense organs;
skin; stomatognathic system; unclassified/mixed; or urinary tract. Template sequences, component
sequences, and cDNA library/tissue information are found in the LIFESEQ GOLD database (Incyte
Genomics, Palo Alto CA).

Table 5 shows the tissue distribution profile for the templates of the invention. For each template, the three most frequently observed tissue categories are shown in column 3, along with the

percentage of component sequences belonging to each category. Only tissue categories with percentage values of  $\geq 10\%$  are shown. A tissue distribution of "widely distributed" in column 3 indicates percentage values of <10% in all tissue categories.

# VII. Transcript Image Analysis

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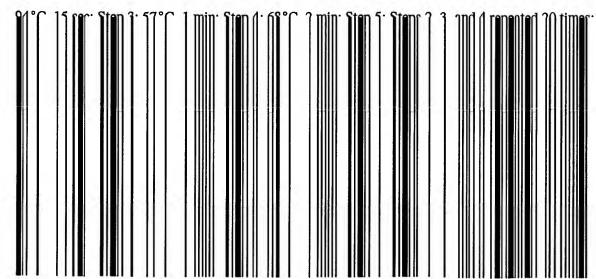
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Transcript images are generated as described in Seilhamer et al., "Comparative Gene Transcript Analysis," U.S. Patent Number 5,840,484, incorporated herein by reference.

# VIII. Extension of Polynucleotide Sequences and Isolation of a Full-length cDNA

Oligonucleotide primers designed using an mddt of the Sequence Listing are used to extend the nucleic acid sequence. One primer is synthesized to initiate 5' extension of the template, and the other primer, to initiate 3' extension of the template. The initial primers may be designed using OLIGO 4.06 software (National Biosciences, Inc. (National Biosciences), Plymouth MN), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations are avoided. Selected human cDNA libraries are used to extend the sequence. If more than one extension is necessary or desired, additional or nested sets of primers are designed.

High fidelity amplification is obtained by PCR using methods well known in the art. PCR is performed in 96-well plates using the PTC-200 thermal cycler (MJ Research). The reaction mix contains DNA template, 200 nmol of each primer, reaction buffer containing Mg<sup>2+</sup>, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, and ß-mercaptoethanol, Taq DNA polymerase (Amersham Pharmacia Biotech), ELONGASE enzyme (Life Technologies), and Pfu DNA polymerase (Stratagene), with the following parameters for primer pair PCI A and PCI B: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C. In the alternative, the parameters for primer pair T7 and SK+ are as follows: Step 1: 94°C, 3 min; Step 2:



to determine which reactions are successful in extending the sequence.

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The extended nucleotides are desalted and concentrated, transferred to 384-well plates, digested with CviJI cholera virus endonuclease (Molecular Biology Research, Madison WI), and sonicated or sheared prior to religation into pUC 18 vector (Amersham Pharmacia Biotech). For shotgun sequencing, the digested nucleotides are separated on low concentration (0.6 to 0.8%) agarose gels, fragments are excised, and agar digested with AGAR ACE (Promega). Extended clones are religated using T4 ligase (New England Biolabs, Inc., Beverly MA) into pUC 18 vector (Amersham Pharmacia Biotech), treated with Pfu DNA polymerase (Stratagene) to fill-in restriction site overhangs, and transfected into competent <u>E. coli</u> cells. Transformed cells are selected on antibiotic-containing media, individual colonies are picked and cultured overnight at 37°C in 384-well plates in LB/2x carbenicillin liquid media.

The cells are lysed, and DNA is amplified by PCR using Taq DNA polymerase (Amersham Pharmacia Biotech) and Pfu DNA polymerase (Stratagene) with the following parameters: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 72°C, 2 min; Step 5: steps 2, 3, and 4 repeated 29 times; Step 6: 72°C, 5 min; Step 7: storage at 4°C. DNA is quantified by PICOGREEN reagent (Molecular Probes) as described above. Samples with low DNA recoveries are reamplified using the same conditions as described above. Samples are diluted with 20% dimethysulfoxide (1:2, v/v), and sequenced using DYENAMIC energy transfer sequencing primers and the DYENAMIC DIRECT kit (Amersham Pharmacia Biotech) or the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Applied Biosystems).

In like manner, the mddt is used to obtain regulatory sequences (promoters, introns, and enhancers) using the procedure above, oligonucleotides designed for such extension, and an appropriate genomic library.

#### 25 IX. Labeling of Probes and Southern Hybridization Analyses

Hybridization probes derived from the mddt of the Sequence Listing are employed for screening cDNAs, mRNAs, or genomic DNA. The labeling of probe nucleotides between 100 and 1000 nucleotides in length is specifically described, but essentially the same procedure may be used with larger cDNA fragments. Probe sequences are labeled at room temperature for 30 minutes using a T4 polynucleotide kinase,  $\gamma^{32}$ P-ATP, and 0.5X One-Phor-All Plus (Amersham Pharmacia Biotech) buffer and purified using a ProbeQuant G-50 Microcolumn (Amersham Pharmacia Biotech). The probe mixture is diluted to  $10^7$  dpm/ $\mu$ g/ml hybridization buffer and used in a typical membrane-based hybridization analysis.

The DNA is digested with a restriction endonuclease such as Eco RV and is electrophoresed

through a 0.7% agarose gel. The DNA fragments are transferred from the agarose to nylon membrane (NYTRAN Plus, Schleicher & Schuell, Inc., Keene NH) using procedures specified by the manufacturer of the membrane. Prehybridization is carried out for three or more hours at 68°C, and hybridization is carried out overnight at 68°C. To remove non-specific signals, blots are sequentially washed at room temperature under increasingly stringent conditions, up to 0.1x saline sodium citrate (SSC) and 0.5% sodium dodecyl sulfate. After the blots are placed in a PHOSPHORIMAGER cassette (Molecular Dynamics) or are exposed to autoradiography film, hybridization patterns of standard and experimental lanes are compared. Essentially the same procedure is employed when screening RNA.

#### 10 X. Chromosome Mapping of mddt

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The cDNA sequences which were used to assemble SEQ ID NO:1-45 are compared with sequences from the Incyte LIFESEQ database and public domain databases using BLAST and other implementations of the Smith-Waterman algorithm. Sequences from these databases that match SEO ID NO:1-45 are assembled into clusters of contiguous and overlapping sequences using assembly algorithms such as PHRAP (Table 7). Radiation hybrid and genetic mapping data available from public resources such as the Stanford Human Genome Center (SHGC), Whitehead Institute for Genome Research (WIGR), and Généthon are used to determine if any of the clustered sequences have been previously mapped. Inclusion of a mapped sequence in a cluster will result in the assignment of all sequences of that cluster, including its particular SEQ ID NO:, to that map location. The genetic map locations of SEQ ID NO:1-45 are described as ranges, or intervals, of human chromosomes. The map position of an interval, in centiMorgans, is measured relative to the terminus of the chromosome's parm. (The centiMorgan (cM) is a unit of measurement based on recombination frequencies between chromosomal markers. On average, 1 cM is roughly equivalent to 1 megabase (Mb) of DNA in humans, although this can vary widely due to hot and cold spots of recombination.) The cM distances are based on genetic markers mapped by Généthon which provide boundaries for radiation hybrid markers whose sequences were included in each of the clusters.

### XI. Microarray Analysis

#### Probe Preparation from Tissue or Cell Samples

Total RNA is isolated from tissue samples using the guanidinium thiocyanate method and polyA<sup>+</sup> RNA is purified using the oligo (dT) cellulose method. Each polyA<sup>+</sup> RNA sample is reverse transcribed using MMLV reverse-transcriptase, 0.05 pg/µ1 oligo-dT primer (21mer), 1X first strand buffer, 0.03 units/µ1 RNase inhibitor, 500 µM dATP, 500 µM dGTP, 500 µM dTTP, 40 µM dCTP, 40 µM dCTP, 40 µM dCTP-Cy3 (BDS) or dCTP-Cy5 (Amersham Pharmacia Biotech). The reverse transcription

reaction is performed in a 25 ml volume containing 200 ng polyA<sup>+</sup> RNA with GEMBRIGHT kits (Incyte). Specific control polyA<sup>+</sup> RNAs are synthesized by in vitro transcription from non-coding yeast genomic DNA (W. Lei, unpublished). As quantitative controls, the control mRNAs at 0.002 ng, 0.02 ng, 0.2 ng, and 2 ng are diluted into reverse transcription reaction at ratios of 1:100,000, 1:10,000, 1:1000 (w/w) to sample mRNA respectively. The control mRNAs are diluted into reverse transcription reaction at ratios of 1:3, 3:1, 1:10, 10:1, 1:25, 25:1 (w/w) to sample mRNA differential expression patterns. After incubation at 37°C for 2 hr, each reaction sample (one with Cy3 and another with Cy5 labeling) is treated with 2.5 ml of 0.5M sodium hydroxide and incubated for 20 minutes at 85°C to the stop the reaction and degrade the RNA. Probes are purified using two successive CHROMA SPIN 30 gel filtration spin columns (CLONTECH Laboratories, Inc. (CLONTECH), Palo Alto CA) and after combining, both reaction samples are ethanol precipitated using 1 ml of glycogen (1 mg/ml), 60 ml sodium acetate, and 300 ml of 100% ethanol. The probe is then dried to completion using a SpeedVAC (Savant Instruments Inc., Holbrook NY) and resuspended in 14 μl 5X SSC/0.2% SDS.

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### Microarray Preparation

Sequences of the present invention are used to generate array elements. Each array element is amplified from bacterial cells containing vectors with cloned cDNA inserts. PCR amplification uses primers complementary to the vector sequences flanking the cDNA insert. Array elements are amplified in thirty cycles of PCR from an initial quantity of 1-2 ng to a final quantity greater than 5  $\mu$ g. Amplified array elements are then purified using SEPHACRYL-400 (Amersham Pharmacia Biotech).

Purified array elements are immobilized on polymer-coated glass slides. Glass microscope slides (Corning) are cleaned by ultrasound in 0.1% SDS and acetone, with extensive distilled water washes between and after treatments. Glass slides are etched in 4% hydrofluoric acid (VWR Scientific Products Corporation (VWR), West Chester, PA), washed extensively in distilled water, and coated with 0.05% aminopropyl silane (Sigma) in 95% ethanol. Coated slides are cured in a 110°C oven.

Array elements are applied to the coated glass substrate using a procedure described in US Patent No. 5,807,522, incorporated herein by reference. 1  $\mu$ l of the array element DNA, at an average concentration of 100 ng/ $\mu$ l, is loaded into the open capillary printing element by a high-speed robotic apparatus. The apparatus then deposits about 5 nl of array element sample per slide.

Microarrays are UV-crosslinked using a STRATALINKER UV-crosslinker (Stratagene). Microarrays are washed at room temperature once in 0.2% SDS and three times in distilled water. Non-specific binding sites are blocked by incubation of microarrays in 0.2% casein in phosphate buffered saline (PBS) (Tropix, Inc., Bedford, MA) for 30 minutes at 60°C followed by washes in 0.2%

SDS and distilled water as before.

#### Hybridization

Hybridization reactions contain 9  $\mu$ 1 of probe mixture consisting of 0.2  $\mu$ g each of Cy3 and Cy5 labeled cDNA synthesis products in 5X SSC, 0.2% SDS hybridization buffer. The probe mixture is heated to 65°C for 5 minutes and is aliquoted onto the microarray surface and covered with an 1.8 cm² coverslip. The arrays are transferred to a waterproof chamber having a cavity just slightly larger than a microscope slide. The chamber is kept at 100% humidity internally by the addition of 140  $\mu$ 1 of 5x SSC in a corner of the chamber. The chamber containing the arrays is incubated for about 6.5 hours at 60°C. The arrays are washed for 10 min at 45°C in a first wash buffer (1X SSC, 0.1% SDS), three times for 10 minutes each at 45°C in a second wash buffer (0.1X SSC), and dried.

#### **Detection**

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Reporter-labeled hybridization complexes are detected with a microscope equipped with an Innova 70 mixed gas 10 W laser (Coherent, Inc., Santa Clara CA) capable of generating spectral lines at 488 nm for excitation of Cy3 and at 632 nm for excitation of Cy5. The excitation laser light is focused on the array using a 20X microscope objective (Nikon, Inc., Melville NY). The slide containing the array is placed on a computer-controlled X-Y stage on the microscope and raster-scanned past the objective. The 1.8 cm x 1.8 cm array used in the present example is scanned with a resolution of 20 micrometers.

In two separate scans, a mixed gas multiline laser excites the two fluorophores sequentially. Emitted light is split, based on wavelength, into two photomultiplier tube detectors (PMT R1477, Hamamatsu Photonics Systems, Bridgewater NJ) corresponding to the two fluorophores. Appropriate filters positioned between the array and the photomultiplier tubes are used to filter the signals. The emission maxima of the fluorophores used are 565 nm for Cy3 and 650 nm for Cy5. Each array is typically scanned twice, one scan per fluorophore using the appropriate filters at the laser source, although the apparatus is capable of recording the spectra from both fluorophores simultaneously.

The sensitivity of the scans is typically calibrated using the signal intensity generated by a cDNA control species added to the probe mix at a known concentration. A specific location on the array contains a complementary DNA sequence, allowing the intensity of the signal at that location to be correlated with a weight ratio of hybridizing species of 1:100,000. When two probes from different sources (e.g., representing test and control cells), each labeled with a different fluorophore, are hybridized to a single array for the purpose of identifying genes that are differentially expressed, the

calibration is done by labeling samples of the calibrating cDNA with the two fluorophores and adding identical amounts of each to the hybridization mixture.

The output of the photomultiplier tube is digitized using a 12-bit RTI-835H analog-to-digital (A/D) conversion board (Analog Devices, Inc., Norwood, MA) installed in an IBM-compatible PC computer. The digitized data are displayed as an image where the signal intensity is mapped using a linear 20-color transformation to a pseudocolor scale ranging from blue (low signal) to red (high signal). The data is also analyzed quantitatively. Where two different fluorophores are excited and measured simultaneously, the data are first corrected for optical crosstalk (due to overlapping emission spectra) between the fluorophores using each fluorophore's emission spectrum.

A grid is superimposed over the fluorescence signal image such that the signal from each spot is centered in each element of the grid. The fluorescence signal within each element is then integrated to obtain a numerical value corresponding to the average intensity of the signal. The software used for signal analysis is the GEMTOOLS gene expression analysis program (Incyte).

#### 15 XII. Complementary Nucleic Acids

Sequences complementary to the mddt are used to detect, decrease, or inhibit expression of the naturally occurring nucleotide. The use of oligonucleotides comprising from about 15 to 30 base pairs is typical in the art. However, smaller or larger sequence fragments can also be used. Appropriate oligonucleotides are designed from the mddt using OLIGO 4.06 software (National Biosciences) or other appropriate programs and are synthesized using methods standard in the art or ordered from a commercial supplier. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent transcription factor binding to the promoter sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding and processing of the transcript.

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#### XIII. Expression of MDDT

Expression and purification of MDDT is accomplished using bacterial or virus-based expression systems. For expression of MDDT in bacteria, cDNA is subcloned into an appropriate vector containing an antibiotic resistance gene and an inducible promoter that directs high levels of cDNA transcription. Examples of such promoters include, but are not limited to, the *trp-lac* (*tac*) hybrid promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory element. Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3). Antibiotic resistant bacteria express MDDT upon induction with isopropyl beta-D-thiogalactopyranoside (IPTG). Expression of MDDT in eukaryotic cells is achieved by infecting insect

or mammalian cell lines with recombinant <u>Autographica californica</u> nuclear polyhedrosis virus (AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is replaced with cDNA encoding MDDT by either homologous recombination or bacterial-mediated transposition involving transfer plasmid intermediates. Viral infectivity is maintained and the strong polyhedrin promoter drives high levels of cDNA transcription. Recombinant baculovirus is used to infect <u>Spodoptera frugiperda</u> (Sf9) insect cells in most cases, or human hepatocytes, in some cases. Infection of the latter requires additional genetic modifications to baculovirus. (See e.g., Engelhard, <u>supra</u>; and Sandig, <u>supra</u>.)

In most expression systems, MDDT is synthesized as a fusion protein with, e.g., glutathione S-transferase (GST) or a peptide epitope tag, such as FLAG or 6-His, permitting rapid, single-step, affinity-based purification of recombinant fusion protein from crude cell lysates. GST, a 26-kilodalton enzyme from Schistosoma japonicum, enables the purification of fusion proteins on immobilized glutathione under conditions that maintain protein activity and antigenicity (Amersham Pharmacia Biotech). Following purification, the GST moiety can be proteolytically cleaved from MDDT at specifically engineered sites. FLAG, an 8-amino acid peptide, enables immunoaffinity purification using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman Kodak Company, Rochester NY). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins (QIAGEN). Methods for protein expression and purification are discussed in Ausubel (1995, supra, Chapters 10 and 16). Purified MDDT obtained by these methods can be used directly in the following activity assay.

# XIV. Demonstration of MDDT Activity

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MDDT, or biologically active fragments thereof, are labeled with <sup>125</sup>I Bolton-Hunter reagent. (See, e.g., Bolton, A.E. and W.M. Hunter (1973) Biochem. J. 133:529-539.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled MDDT, washed, and any wells with labeled MDDT complex are assayed. Data obtained using different concentrations of MDDT are used to calculate values for the number, affinity, and association of MDDT with the candidate molecules.

Alternatively, molecules interacting with MDDT are analyzed using the yeast two-hybrid system as described in Fields, S. and O. Song (1989) Nature 340:245-246, or using commercially available kits based on the two-hybrid system, such as the MATCHMAKER system (CLONTECH).

MDDT may also be used in the PATHCALLING process (CuraGen Corp., New Haven CT) which employs the yeast two-hybrid system in a high-throughput manner to determine all interactions

between the proteins encoded by two large libraries of genes (Nandabalan, K. et al. (2000) U.S. Patent No. 6,057,101).

### XV. Functional Assays

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MDDT function is assessed by expressing mddt at physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a mammalian expression vector containing a strong promoter that drives high levels of cDNA expression. Vectors of choice include pCMV SPORT (Life Technologies) and pCR3.1 (Invitrogen Corporation, Carlsbad CA), both of which contain the cytomegalovirus promoter.  $5-10~\mu g$  of recombinant vector are transiently transfected into a human cell line, preferably of endothelial or hematopoietic origin, using either liposome formulations or electroporation.  $1-2~\mu g$  of an additional plasmid containing sequences encoding a marker protein are co-transfected.

Expression of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector.

Marker proteins of choice include, e.g., Green Fluorescent Protein (GFP; CLONTECH), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an automated laser optics-based technique, is used to identify transfected cells expressing GFP or CD64-GFP and to evaluate the apoptotic state of the cells and other cellular properties.

FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide; changes in cell size and granularity as measured by forward light scatter and 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in expression of cell surface and intracellular proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are discussed in Ormerod, M. G. (1994) Flow Cytometry, Oxford, New York NY.

The influence of MDDT on gene expression can be assessed using highly purified populations of cells transfected with sequences encoding MDDT and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected cells using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Inc., Lake Success NY). mRNA can be purified from the cells using methods well known by those of skill in the art.

Expression of mRNA encoding MDDT and other genes of interest can be analyzed by northern analysis or microarray techniques.

#### XVI. Production of Antibodies

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MDDT substantially purified using polyacrylamide gel electrophoresis (PAGE; see, e.g., Harrington, M.G. (1990) Methods Enzymol. 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

Alternatively, the MDDT amino acid sequence is analyzed using LASERGENE software (DNASTAR) to determine regions of high immunogenicity, and a corresponding peptide is synthesized and used to raise antibodies by means known to those of skill in the art. Methods for selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel, 1995, supra, Chapter 11.)

Typically, peptides 15 residues in length are synthesized using an ABI 431A peptide synthesizer (Applied Biosystems) using fmoc-chemistry and coupled to KLH (Sigma) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase immunogenicity. (See, e.g., Ausubel, <a href="mailto:supra">supra</a>.) Rabbits are immunized with the peptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for antipeptide activity by, for example, binding the peptide to plastic, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG. Antisera with antipeptide activity are tested for anti-MDDT activity using protocols well known in the art, including ELISA, RIA, and immunoblotting.

#### XVII. Purification of Naturally Occurring MDDT Using Specific Antibodies

Naturally occurring or recombinant MDDT is substantially purified by immunoaffinity chromatography using antibodies specific for MDDT. An immunoaffinity column is constructed by covalently coupling anti-MDDT antibody to an activated chromatographic resin, such as CNBr-activated SEPHAROSE (Amersham Pharmacia Biotech). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing MDDT are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of MDDT (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/MDDT binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and MDDT is collected.

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the above-described modes for carrying out the invention which are obvious to those skilled in the field of molecular biology or related fields are intended to be within the scope of the following claims.

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		TABLE 1		
SEQ ID NO:	: Template ID	GI Number	Probability Score	Annotation
-	LG:977683.1:2000FEB18	g10764778		phospholnosital 3-phosphata-binding protain-9 (Hamo
8	LG:893050.1:2000FEB18	06634025	2.00E-81	KIAA0379 profein (Homo sablens)
က	LG:980153.1:2000FEB18	g7263990	0	dJ93K22.1 (novel protein (contains DKFZP564B116)) (Homo
				sapiens)
4	LG:350398.1:2000FEB18	g3882175	3.00E-10	KIAA0727 protein (Homo sapiens)
2	LG:475551.1:2000FEB18	g861029	0	SH3 domain binding protein (Mus musculus)
9	LG:481407.2:2000FEB18	g6119546	1.00E-41	hypothetical protein; 114721-113936 (Arabidopsis thalland)
7	LI:443580.1:2000FEB01	94589566	3.00E-34	KIAA0961 protein (Homo sapiens)
80	LI:803015.1:2000FEB01	g5262560	2.00E-35	hypothetical protein (Homo saplens)
O	LG:027410.3:2000MAY19	g10438267	1.00E-65	unnamed protein product (Homo sapiens)
<del>1</del>	LG:171377.1:2000MAY19	g3077703	1.00E-107	mitsugumin29 (Oryctolagus cuniculus)
Ξ	LG:352559.1:2000MAY19	g7243243	2.00E-43	KIAA1431 protein (Homo saplens)
12	LG:247384.1:2000MAY19	g9945010	1.00E-118	RING-finger protein MURF (Mus musculus)
13	LG:403872.1:2000MAY19	g7020303	0	unnamed protein product (Homo sapiens)
4	LG:1135213.1:2000MAY19	g6692607	2.00E-65	MGA protein (Mus musculus)
15	LG:474284.2:2000MAY19	g1488047	2.00E-30	RING finger protein (Xenopus laevis)
16	LG:342147.1:2000MAY19	g2477511	3.00E-41	Homo sapiens p20 protein (pir B53814)
17	LG:1097300.1:2000MAY19	g2078531	1.00E-70	Mark (Mus musculus)
<del>1</del> 8	LG:444850.9:2000MAY19	g199000	0	interferon-gamma inducible protein (Mus musculus)
19	LG:402231.6:2000MAY19	g7020737	6.00E-77	unnamed profein product (Homo sapiens)
20	LG:1076157.1:2000MAY19	g5262560	3.00E-65	hypothetical protein (Homo sapiens)
21	LG:1083142.1:2000MAY19	g4589566	3.00E-23	KIAA0961 protein (Homo sapiens)
22	LG:1083264.1:2000MAY19	g10047297	2.00E-25	KIAA1611 protein (Homo sapiens)
83	LG:350793.2:2000MAY19	g7242973	0	KIAA1309 protein (Homo sapiens)
54	LG:408751.3:2000MAY19	g8886025	1.00E-134	collapsin response mediator protein-5 (Homo saplens)
52	LI:336120.1:2000MAY01	g1864085	1.00E-160	glypican-5 (Homo sapiens)
56	LI:234104.2:2000MAY01	g1518505	1.00E-114	G-protein coupled inwardly rectifying K+ channel (Mus
27	LI:450887.1:2000MAY01	g7629994	3.00E-34	musculus) 60S RIBOSOMAL PROTEIN L36 homolog (Arabidopsis
Ş			•	thaliana)
8 8	LI:119992.3:2000MAY01	g/243089 n7263990		KIAA1354 protein (Homo sapiens) d 193K22 1 (noviel protein (contains DKF2P564B114)) (Homo
30	LI:406860.20:2000MAY01	g10435919	3.00E-57	unnamed protein product (Homo sapiens)

saplens) unnamed protein product (Homo saplens)	2.00E-62	g7022971	LI:252904.5:2000MAY01	45
SH3 and PX domain-containing protein SH3PX1 (Homo	1.00E-63	g6164628	LI:236386.4:2000MAY01	4
TLR6 (Mus musculus)	0	g5006250	LI:007302.1:2000MAY01	43
protocadherin alpha 7 short form protein (Homo sapiens)	0	g5457019	LI:1165828.1:2000MAY01	42
protocadherin beta 12 (Homo sapiens)	0	g5457031	LI:1084246.1:2000MAY01	4
32 kd accessory protein (Bos taurus)	2.00E-74	g736727	LI:023518.3:2000MAY01	9
KIAA1311 protein (Homo sapiens)	2.00E-51	g7242977	LI:000290.1:2000MAY01	33
transient receptor potential 4 (Homo saplens)	0	g5802615	LI:817314.1:2000MAY01	38
ACE-related carboxypeptidase ACE2 (Homo sapiens)	1.00E-101	g9802433	LI:347572.1:2000MAY01	37
KIAA1223 protein (Homo sapiens)	0	g6330617	LI:334386.1:2000MAY01	36
BC85722_1 (Homo saplens)	0	g4210501	LI:243660.4:2000MAY01	32
KIAA0780 protein (Homo sapiens)	7.00E-79	g3882281	LI:1144066.1:2000MAY01	34.
unnamed protein product (Mus musculus)	1.00E-116	g7670362	LI:757439.1:2000MAY01	ဗ္ဗ
F02569_2 (Homo saplens)	1.00E-106	g3184264	LI:895427.1:2000MAY01	35
unnamed protein product (Homo sapiens)	1.00E-131	g10436290	LI:142384.1:2000MAY01	9

			;	TABLE 2	į		
SEG ID NO:		Start	Stop		Pfam Hiit	Pfam Description	E-value
- ,	LG:977683.1:2000FEB18	540	695		H	PH domain	6.70E-11
-	LG:977683.1:2000FEB18	204	293	forward 3	<b>*</b>	WW domain	7.50E-05
01	LG:893050.1:2000FEB18	211	308	forward 1	ank	Ank repeat	1.60E-05
က	LG:980153.1:2000FEB18	754	852	forward 1	ank	Ank repeat	8.00E-04
က	LG:980153.1:2000FEB18	2131	2565	forward 1	втв	BTB/POZ domain	6.90E-07
က	LG:980153.1:2000FEB18	1084	1239	forward 1	. RCC1	Regulator of chromosome condensation	n 3.70E-04
4	LG:350398.1:2000FEB18	7	123	forward 1	myosin_head	Myosin head (motor domain)	2.60E-16
2	LG:475551.1:2000FEB18	705	1157	forward 3	RhoGAP	RhoGAP domain	8.10E-71
9	LG:481407.2:2000FEB18	225	440	forward 3	E	RNA recognition motif. (a.k.a. RRM, RBE 1.50E-22	JC 1.50E-22
9	LG:481407.2:2000FEB18	504	557	forward 3	zf-CCHC	Zinc knuckle	7.00E-04
7	LI:443580.1:2000FEB01	262	450	forward 1	KRAB	KRAB box	1.60E-41
7	LI:443580.1:2000FEB01	. 625	693	forward 1	zf-C2H2	Zinc finger, C2H2 type	2.20E-06
80	LI:803015.1:2000FEB01	159	599	forward 3	KRAB	KRAB box	2.30E-17
0	LG:027410.3:2000MAY19	177	290	forward 3	WD40	WD domain, G-beta repeat	6.20E-06
<b>1</b> 0	LG:171377.1:2000MAY19	300	848	forward 3	Synaptophysin	Synaptophysin / synaptoporin	2.10E-20
=	LG:352559.1:2000MAY19	125	313	forward 2	KRAB	KRAB box	1.60E-41
52	LG:247384.1:2000MAY19	182	256	forward 2	zf-C3HC4	Zinc finger, C3HC4 type (RING finger)	1.80E-06
13	LG:403872.1:2000MAY19	717	1187	forward 3	PAP2	PAP2 superfamily	1.80E-09
4	LG:1135213.1:2000MAY19	340	531	forward 1	T-box	T-box	8.80E-27
15	LG:474284.2:2000MAY19	73	195	forward 1	zf-C3HC4	Zinc finger, C3HC4 type (RING finger)	1.20E-13
16	LG:342147.1:2000MAY19	290	469	forward 2	crystallin	Alpha crystallin A chain, N terminal	3.10E-09
<del>1</del> 6	LG:342147.1:2000MAY19	452	. 628	forward 2	HSP20	Hsp20/alpha crystallin family	7.20E-12
17	LG:1097300.1:2000MAY19	29	250	forward 2	EE.	RNA recognition motif. (a.k.a. RRM, RBL 4.10E-16	£ 4.10E-16
<del>1</del> 8	LG:444850.9:2000MAY19	190	1290	forward 1	GBP	Guanylate-binding protein	4.20E-247
19	LG:402231.6:2000MAY19	258	380	forward 3	zf-C3HC4	Zinc finger, C3HC4 type (RING finger)	4.30E-05
20	LG:1076157.1:2000MAY19	180	320	forward 3	KRAB	KRAB box	3.40E-18
27	LG:1083142.1:2000MAY19	129	320	forward 3	KRAB	KRAB box	2.00E-42
55	LG:1083264.1:2000MAY19	440	628	forward 2	KRAB	KRAB box	2.30E-33
23	LG:350793.2:2000MAY19	220	722	forward 3	Kelch	Kelch motif	2.70E-11
24	LG:408751.3:2000MAY19	194	1051	forward 2	Dihydrooratase	Dihydroorotase-like	5.50E-07
25	LI:336120.1:2000MAY01	232	1398	forward 1	Glypican	Glypican	9.90E-141
25	LI:336120.1:2000MAY01	1476	1907	forward 3	Glypican	Glypican	8.60E-70
22	LI:336120.1:2000MAY01	203	775	forward 2	Glypican	Glypican	3.50E-46
56	LI:234104.2:2000MAY01	2517	3002	forward 3	XE.	Inward rectifier potassium channel	8.70E-111

56	LI:234104.2:2000MAY01	2965	3507	forward 1	ЯК	Inward rectifier potassium channel	9.20E-111
27	LI:450887.1:2000MAY01	48	344	forward 3	Ribosomal_L36e	Ribosomal protein L36e	6.90E-41
<b>58</b>	LI:119992.3:2000MAY01	788	925	forward 2	Kelch	Kelch motif	1.50E-09
53	LI:197241.2:2000MAY01	1243	1407	forward 1	RCC1	Regulator of chromosome condensation	1.60E-04
30	LI:406860.20:2000MAY01	228	407	forward 3	<u>ģ</u>	Immunoglobulin domain	1.90E-08
31	LI:142384.1:2000MAY01	318	791	forward 3	UQ_con	Ubiquitin-conjugating enzyme	1.40E-16
35	Lf:895427.1:2000MAY01	437	206	forward 2	RhoGAP	RhoGAP domain	1.20E-40
33	LI:757439.1:2000MAY01	1040	1162	forward 2	zf-C3HC4	Zinc finger, C3HC4 type (RING finger)	7.20E-10
34	LI:1144066.1:2000MAY01	222	365	forward 3	Nįmį	jmjN domain	2.80E-23
32	LI:243660.4:2000MAY01	316	522	forward 1	HMG_box	HMG (high mobility group) box	8.60E-17
36	LI:334386.1:2000MAY01	272	370	forward 2	ank	Ank repeat	4.90E-08
36	LI:334386.1:2000MAY01	735	833	forward 3	ank	Ank repeat	4.50E-05
37	LI:347572.1:2000MAY01	130	1878	forward 1	Peptidase_M2	Angiotensin-converting enzyme	2.60E-05
38	LI:817314.1:2000MAY01	934	2034	forward 1	Trans_recep	Transient receptor	6.50E-260
38	Lf:817314.1:2000MAY01	1929	2321	forward 3	Trans_recep	Transient receptor	2.20E-81
39	LI:000290.1:2000MAY01	960	1040	forward 3	zf-CCCH	Zinc finger C-x8-C-x5-C-x3-H type (and £7.70E-04	£7.70E-04
40	LI:023518.3:2000MAY01	195	845	forward 3	vATP-synt_AC39	ATP synthase (C/AC39) subunit	5.30E-38
41	LI:1084246.1:2000MAY01	1443	1733	forward 3	cadherin	Cadherin domain	2.30E-20
4	LI:1084246.1:2000MAY01	875	1150	forward 2	cadherin	Cadherin domain	6.60E-17
42	LI:1165828.1:2000MAY01	1421	1705	forward 2	cadherin	Cadherin domain	1.30E-19
43	LI:007302.1:2000MAY01	1646	1810	forward 2	LRRCT	Leucine rich repeat C-terminal domain	2.60E-13
43	LI:007302.1:2000MAY01	1991	2455	forward 2	TIR	TIR domain	3.50E-37
44	LI:236386.4:2000MAY01	229	820	forward 2	SH3	SH3 domain	5.20E-07
45	LI:252904.5:2000MAY01	358	495	forward 1	Kelch	Kelch motif	3.80E-07

TΔ	RI	E	3
-	01		

			TABLE 3			
SEQ ID NO:	Template ID	Start	Stop	Frame	Domain	Topology
	1.0.00000000000000000000000000000000000		450	fam	Type	A1 :
1	LG:977683.1:2000FEB18	373	459	forward 1	TM	N in
1	LG:977683.1:2000FEB18	657	731	forward 3	TM	N out
2	LG:893050.1:2000FEB18	15	101	forward 3	TM	N out
3	LG:980153.1:2000FEB18	313	375	forward 1	TM	N out
3	LG:980153.1:2000FEB18	391	453	forward 1	TM	N out
3	LG:980153.1:2000FEB18	278	364	forward 2	TM	N out
3	LG:980153.1:2000FEB18	416	493	forward 2	TM	N out
3	LG:980153.1:2000FEB18	809	871	forward 2	TM	N out
3	LG:980153.1:2000FEB18	902	964	forward 2	TM	N out
3	LG:980153.1:2000FEB18	1181	1264	forward 2	TM	N out
3	LG:980153.1:2000FEB18		1510	forward 2	TM	N out
3	LG:980153.1:2000FEB18	1733	1798	forward 2	TM	N out
3	LG:980153.1:2000FEB18		1954	forward 2	TM	N out
3	LG:980153.1:2000FEB18	_	2227	forward 2	TM	N out
3	LG:980153.1:2000FEB18	2261	2308	forward 2	TM	N out
3	LG:980153.1:2000FEB18	60	125	forward 3	TM	N in
3	LG:980153.1:2000FEB18	402	476	forward 3	TM	N in
3	LG:980153.1:2000FEB18	2031		forward 3	TM	N in
3	LG:980153.1:2000FEB18		2213	forward 3	TM	N in
5	LG:475551.1:2000FEB18		2208	forward 1	TM	N in
5	LG:475551.1:2000FEB18		2125	forward 2	TM	N out
5	LG:475551.1:2000FEB18		1217	forward 3	TM	N in
6	LG:481407.2:2000FEB18	874	927	forward 1	TM	
6	LG:481407.2:2000FEB18	949	1035	forward 1	TM	
6	LG:481407.2:2000FEB18	1081	1161	forward 1	TM	
6	LG:481407.2:2000FEB18	1510		forward 1	TM	
6	LG:481407.2:2000FEB18	1355		forward 2		N out
6	LG:481407.2:2000FEB18	1439		forward 2		N out
6	LG:481407.2:2000FEB18	1326		forward 3		N in
6	LG:481407.2:2000FEB18		1526	forward 3		N in
6	LG:481407.2:2000FEB18	1545		forward 3		N in
7	LI:443580.1:2000FEB01	488	574	forward 2		N out
10	LG:171377.1:2000MAY19	318	386	forward 3		N in
10	LG:171377.1:2000MAY19	549	635	forward 3		N in
10	LG:171377.1:2000MAY19	669	740	forward 3		N in
12	LG:247384.1:2000MAY19	1381	1461	forward 1	TM	N in
12	LG:247384.1:2000MAY19	1624		forward 1	TM	N in
12	LG:247384.1:2000MAY19	1409		forward 2		N in
12	LG:247384.1:2000MAY19	1395		forward 3		N in
12	LG:247384.1:2000MAY19	1617		forward 3		N in
13	LG:403872.1:2000MAY19	535	621	forward 1	TM	N in
13	LG:403872.1:2000MAY19	1360		forward 1	TM	N in
13	LG:403872.1:2000MAY19	1522		forward 1	TM	N in
13	LG:403872.1:2000MAY19	1828		forward 1	TM	N in
13	LG:403872.1:2000MAY19		2022	forward 1	TM	N in
13	LG:403872.1:2000MAY19	299	349	forward 2		N in
13	LG:403872.1:2000MAY19	1361	1423	forward 2		N in
13	LG:403872.1:2000MAY19	1439		forward 2		N in
13	LG:403872.1:2000MAY19	1553		forward 2		N in
13	LG:403872.1:2000MAY19	1859		forward 2		N in
13	LG:403872.1:2000MAY19		2110	forward 2		N in
13	LG:403872.1:2000MAY19	2117		forward 2		N in
13	LG:403872.1:2000MAY19	369	452	forward 3	3 TM	N in
	ĺ	62		1		
				1		

13	LG:403872.1:2000MAY19	549	635	forward 3	TM	N in
13	LG:403872.1:2000MAY19	708	785	forward 3	TM	N in
13	LG:403872.1:2000MAY19	1101	1187	forward 3	TM	N in
13	LG:403872.1:2000MAY19	1419	1505	forward 3	TM	N in
13	LG:403872.1:2000MAY19	1575	1661	forward 3	TM	N in
13	LG:403872.1:2000MAY19	2115	2192	forward 3	TM	N in
13	LG:403872.1:2000MAY19	2226	2273	forward 3	TM	N in
14	LG:1135213.1:2000MAY19	41	127	forward 2	TM	N out
14	LG:1135213.1:2000MAY19	215	274	forward 2	TM	N out
14	LG:1135213.1:2000MAY19	293	379	forward 2	TM	N out
14	LG:1135213.1:2000MAY19	389	475	forward 2	TM	N out
16	LG:342147.1:2000MAY19	142	204	forward 1	TM	N out
16	LG:342147.1:2000MAY19	171	251	forward 3	TM	N out
17	LG:1097300.1:2000MAY19	487	564	forward 1	TM	14 Out
17	LG:1097300.1:2000MAY19	805	891	forward 1	TM	
17	LG:1097300.1:2000MAY19	1372	1458	forward 1	TM	
17	LG:1097300.1:2000MAY19	668	754	forward 2	TM	Marit
17	LG:1097300.1:2000MAY19	803	874	forward 2	TM	N out
17	LG:1097300.1:2000MAY19	1358	1441	forward 2	TM	N out
17	LG:1097300.1:2000MAY19	522	578			N out
17	LG:1097300.1:2000MAY19	750	836	forward 3 forward 3	TM	N in
17	LG:1097300.1:2000MAY19	894	956	_	TM	N in
17	LG:1097300.1:2000MAY19	1068	1145	forward 3	TM	N in
18	LG:444850.9:2000MAY19	253	315	forward 3	TM	N in
19	LG:402231.6:2000MAY19	407	484	forward 1	TM	N in
23	LG:350793.2:2000MAY19	148	222	forward 2	TM	N in
23	LG:350793.2:2000MAY19	316	384	forward 1	TM	N in
23	LG:350793.2:2000MAY19	1144	304 1215	forward 1	TM	N in
23	LG:350793.2:2000MAY19	1231	1215	forward 1	TM	N in
23	LG:350793.2:2000MAY19	1339		forward 1	TM	N in
23	LG:350793.2:2000MAY19	1459	1425	forward 1	TM	N in
23	LG:350793.2:2000MAY19	1582	1521	forward 1	TM	N in
23	LG:350793.2:2000MAY19		1662	forward 1	TM	N in
23	LG:350793.2:2000MAY19	1882	1953	forward 1	TM	N in
23	LG:350793.2:2000MAY19	1514	1600	forward 2	TM	
23	LG:350793.2:2000MAY19	2135	2221	forward 2	TM	
23	LG:350793.2:2000MAY19	1422	1493	forward 3	TM	
24	LG:408751.3:2000MAY19	2268	2354	forward 3	TM	
24	LG:408751.3:2000MAY19	1202	1264	forward 2	TM	N out
25	LI:336120.1:2000MAY01	1137	1223	forward 3	TM	N in
25	LI:336120.1:2000MAY01	241	297	forward 1	TM	N in
25	LI:336120.1:2000MAY01	616	702	forward 1	TM	N in
25	LI:336120.1:2000MAY01	1141	1200	forward 1	TM	N in
25 25	LI:336120.1:2000MAY01	2524	2598	forward 1	TM	N in
25 25	LI:336120.1:2000MAY01	1163	1213	forward 2	TM	N in
25 25	LI:336120.1:2000MAY01	1922	1972	forward 2	TM	N in
25 25		2060	2119	forward 2	TM	N in
25 25	LI:336120.1:2000MAY01 LI:336120.1:2000MAY01	2510	2596	forward 2	TM	N in
		663	749	forward 3	TM	N in
25 25	LI:336120.1:2000MAY01	1380	1445	forward 3	TM	N in
25 25	LI:336120.1:2000MAY01	1839	1925	forward 3	TM	N in
25 25	LI:336120.1:2000MAY01	2148	2234	forward 3	TM	N in
25 25	LI:336120.1:2000MAY01	2418	2471	forward 3	TM	N in
25	LI:336120.1:2000MAY01	2499	2585	forward 3	TM	N in
26	LI:234104.2:2000MAY01	1873	1947	forward 1	TM	N out
26	LI:234104.2:2000MAY01	2155	2241	forward 1	TM	N out
26	LI:234104.2:2000MAY01	3616	3690	forward 1	TM	N out

3

26	LI:234104.2:2000MAY01	1112	1168	forward 2	TM	N in
26	LI:234104.2:2000MAY01	2216	2302	forward 2	TM	N in
26	LI:234104.2:2000MAY01	3632	3718	forward 2	TM	N in
26	LI:234104.2:2000MAY01	3998	4045	forward 2	TM	N in
26	LI:234104.2:2000MAY01	1314	1400	forward 3	TM	N in
26	LI:234104.2:2000MAY01	2172	2258	forward 3	TM	N in
26	LI:234104.2:2000MAY01	2607	2684	forward 3	TM	N in
26	LI:234104.2:2000MAY01	2739	2798	forward 3	TM	N in
26	LI:234104.2:2000MAY01	2841	2891	forward 3	TM	N in
26	LI:234104.2:2000MAY01	3621	3707	forward 3	TM	N in
26	LI:234104.2:2000MAY01	4080	4145	forward 3	TM	N in
28	LI:119992.3:2000MAY01	22	102	forward 1	TM	N out
28	LI:119992.3:2000MAY01	151	237	forward 1	TM	N out
28	LI:119992.3:2000MAY01	1444	1530	forward 1	TM	N out
28	LI:119992.3:2000MAY01	1603	1683	forward 1	TM	N out
28	LI:119992.3:2000MAY01	1729	1809	forward 1	TM	N out
28	LI:119992.3:2000MAY01	2197	2253	forward 1	TM	N out
28	LI:119992.3:2000MAY01	2269	2355	forward 1	TM	N out
28	LI:119992.3:2000MAY01	2989	3075	forward 1	TM	N out
28	LI:119992.3:2000MAY01	3163	3249	forward 1	TM	N out
28	LI:119992.3:2000MAY01	1247	1333	forward 2	TM	N in
28	LI:119992.3:2000MAY01	1538	1606	forward 2	TM	N in
28	LI:119992.3:2000MAY01	2207	2293	forward 2	TM	N in
28	LI:119992.3:2000MAY01	2756	2812	forward 2	TM	N in
28	LI:119992.3:2000MAY01	3098	3169	forward 2	TM	N in
28	LI:119992.3:2000MAY01	3281	3343	forward 2	TM	N in
28	LI:119992.3:2000MAY01	3356	3418	forward 2	TM	N in
28	LI:119992.3:2000MAY01	120	188	forward 3	TM	N in
28	LI:119992.3:2000MAY01	627	689	forward 3	TM	N in
28	LI:119992.3:2000MAY01	708	770	forward 3	TM	N in
28	LI:119992.3:2000MAY01	1425	1511	forward 3	TM	N in
28	LI:119992.3:2000MAY01	1782	1868	forward 3	TM	N in
28	LI:119992.3:2000MAY01 LI:119992.3:2000MAY01	2223	2306	forward 3	TM	N in
28		2757	2843 3113	forward 3	TM	N in
28 28	LI:119992.3:2000MAY01 LI:119992.3:2000MAY01	3027 3213	3275	forward 3	TM	N in
28	LI:119992.3:2000MAY01		3374	forward 3 forward 3	TM TM	N in
29	LI:197241.2:2000MAY01	289	369		TM	N in
29	Li:197241,2:2000MAY01	430	507	forward 1 forward 1	TM	N out N out
29	LI:197241.2:2000MAY01	799	861	forward 1	TM	N out
29	LI:197241.2:2000MAY01	889	951	forward 1	TM	N out
29	LI:197241.2:2000MAY01	1798	1863	forward 1	TM	N out
29	LI:197241.2:2000MAY01	1930	2016	forward 1	TM	N out
29	LI:197241.2:2000MAY01	2101	2148	forward 1	TM	N out
29	LI:197241.2:2000MAY01	2206	2262	forward 1	TM	N out
29	LI:197241.2:2000MAY01	416	499	forward 2	TM	N out
29	LI:197241.2:2000MAY01	812	862	forward 2	TM	N out
29	LI:197241.2:2000MAY01	1226	1309		TM	N out
29	LI:197241.2:2000MAY01	1475	1558	forward 2	ТМ	N out
29	LI:197241.2:2000MAY01	2210	2296	forward 2	TM	N out
29	Li:197241.2:2000MAY01	60	125	forward 3	TM	N in
29	LI:197241.2:2000MAY01	333	395	forward 3	TM	N in
29	LI:197241.2:2000MAY01	441	503	forward 3	TM	N in
29	LI:197241.2:2000MAY01	2223	2300	forward 3	TM	N in
31	LI:142384.1:2000MAY01	367	432	forward 1	TM	N out
31	LI:142384.1:2000MAY01	93	155	forward 3	TM	N out
- •	(	<del>-64</del>				

32	LI:895427.1:2000MAY01	1796 1879	forward 2	TM	N in
32	LI:895427.1:2000MAY01	1656 1724	forward 3	TM	N in
33	LI:757439.1:2000MAY01	253 312	forward 1	TM	N in
33	LI:757439.1:2000MAY01	817 900	forward 1	TM	N in
33	LI:757439.1:2000MAY01	1507 1572	forward 1	TM	N in
33	LI:757439.1:2000MAY01	1615 1677	forward 1	TM	N in
33	LI:757439.1:2000MAY01	1696 1758	forward 1	TM	N in
33	LI:757439.1:2000MAY01	1834 1899	forward 1	TM	N in
33	LI:757439.1:2000MAY01	1969 2043	forward 1	TM	N in
33	LI:757439.1:2000MAY01	2107 2193	forward 1	TM	N in
33	LI:757439.1:2000MAY01	2506 2586	forward 1	TM	N in
33	LI:757439.1:2000MAY01	815 901	forward 2	TM	N out
33	LI:757439.1:2000MAY01	1634 1720	forward 2	TM	N out
33	LI:757439.1:2000MAY01	1796 1882	forward 2	TM	N out
33	LI:757439.1:2000MAY01	1952 2026	forward 2	TM	N out
33	LI:757439.1:2000MAY01	2486 2563	forward 2	TM	N out
33	LI:757439.1:2000MAY01	783 869	forward 3	TM	N in
33	LI:757439.1:2000MAY01	996 1049	forward 3	TM	N in
33	LI:757439.1:2000MAY01	1545 1631	forward 3	TM	N in
33	LI:757439.1:2000MAY01	2115 2174	forward 3	TM	N in
35	LI:243660.4:2000MAY01	1247 1333	forward 2	MT	N in
36	LI:334386.1:2000MAY01	538 621	forward 1	TM	
36	LI:334386.1:2000MAY01	922 1008	forward 1	TM	
36	LI:334386.1:2000MAY01	1087 1173	forward 1	TM	
36	LI:334386.1:2000MAY01	1468 1530	forward 1	TM	
36	LI:334386.1:2000MAY01	1570 1632	forward 1	TM	
36	LI:334386.1:2000MAY01	2731 2802	forward 1	TM	
36	LI:334386.1:2000MAY01	2992 3054	forward 1	TM	
36	LI:334386.1:2000MAY01	3325 3387	forward 1	TM	
36	LI:334386.1:2000MAY01	3406 3468	forward 1	TM	
36	LI:334386.1:2000MAY01	3487 3570	forward 1	TM	
36	Li:334386.1:2000MAY01	3766 3852	forward 1	TM	
36	LI:334386.1:2000MAY01	4006 4077	forward 1	TM	
36	LI:334386.1:2000MAY01	4342 4416	forward 1	TM	
36	LI:334386.1:2000MAY01	4615 4686	forward 1	TM	
36	LI:334386.1:2000MAY01	4747 4833	forward 1	TM	
36	LI:334386.1:2000MAY01	5062 5124	forward 1	TM	
36	LI:334386.1:2000MAY01	5140 5202	forward 1	TM	
36	LI:334386.1:2000MAY01	5227 5289	forward 1	TM	
36	LI:334386.1:2000MAY01	5563 5649	forward 1	TM	
36	LI:334386.1:2000MAY01	1235 1321	forward 2	TM	N in
36	LI:334386.1:2000MAY01	2423 2476	forward 2	TM	N in
36	LI:334386.1:2000MAY01	2702 2764	forward 2	TM	N in
36	LI:334386.1:2000MAY01	2792 2854	forward 2	TM	N in
36	LI:334386.1:2000MAY01	3086 3172	forward 2	TM	N in
36	LI:334386.1:2000MAY01	3302 3355	forward 2	TM	N in
36	LI:334386.1:2000MAY01	3452 3517	forward 2	TM	N in
36	LI:334386.1:2000MAY01	3920 4006	forward 2	TM	N in
36	LI:334386.1:2000MAY01	4064 4144	forward 2	TM	N in
36	Ll:334386.1:2000MAY01	4250 4318	forward 2	TM	N in
36	LI:334386.1:2000MAY01	4331 4402	forward 2	TM	N in
36	LI:334386.1:2000MAY01	4523 4576	forward 2	TM	N in
36	LI:334386.1:2000MAY01	4586 4669	forward 2	TM	N in
36	LI:334386.1:2000MAY01	4772 4855	forward 2	TM	N in
36	LI:334386.1:2000MAY01	5039 5125	forward 2	TM	N in
36	LI:334386.1:2000MAY01	5498 5584	forward 2	TM	N in

36	LI:334386.1:2000MAY01	30	116	forward 3	TM	N in
36	LI:334386.1:2000MAY01	324	380	forward 3	TM	N in
36	LI:334386.1:2000MAY01	387	470	forward 3	TM	N in
36	LI:334386.1:2000MAY01	531	608	forward 3	TM	N in
36	LI:334386.1:2000MAY01	1362	1448	forward 3	TM	N in
36	LI:334386.1:2000MAY01	1539	1625	forward 3	TM	N in
36	LI:334386.1:2000MAY01	2232	2279	forward 3	TM	N in
36	LI:334386.1:2000MAY01	2580	2651	forward 3	TM	N in
36	LI:334386.1:2000MAY01	2757	2822	forward 3	TM	N in
36	LI:334386.1:2000MAY01	2820	2870	forward 3	TM	N in
36	LI:334386.1:2000MAY01	3282	3368	forward 3	TM	N in
36	LI:334386.1:2000MAY01	3510	3596	forward 3	TM	N in
36	LI:334386.1:2000MAY01	3981	4064	forward 3	TM	N in
36	LI:334386.1:2000MAY01	4356	4427	forward 3	TM	N in
36	LI:334386.1:2000MAYQ1	4464	4544	forward 3	TM	N in
36	LI:334386.1:2000MAY01	4959	5024	forward 3	TM	N in
36	LI:334386.1:2000MAY01	5601	5687	forward 3	TM	N in
37	LI:347572.1:2000MAY01	790	876	forward 1	TM	N in
37	LI:347572.1:2000MAY01	1354	1434	forward 1	TM	N in
37	LI:347572.1:2000MAY01	2425	2511	forward 1	TM	N in
37	LI:347572.1:2000MAY01	2599	2685	forward 1	ТМ	N in
37	LI:347572.1:2000MAY01	2686	2757	forward 1	TM	N in
37	LI:347572.1:2000MAY01	3133	3207	forward 1	TM	N in
37	LI:347572.1:2000MAY01	1184	1255	forward 2	TM	14 111
37	Ll:347572.1:2000MAY01	2264	2350	forward 2		
37	LI:347572.1:2000MAY01	2597	2665	forward 2	TM	
37	LI:347572.1:2000MAY01	2942	3028	forward 2	TM	
37	LI:347572.1:2000MAY01	3137	3199	forward 2	TM	
37	LI:347572.1:2000MAY01	3227	3289		TM	
37	LI:347572.1:2000MAY01	129	215	forward 2 forward 3	TM	A1 !
37	LI:347572.1:2000MAY01	969			TM	N in
37	LI:347572.1:2000MAY01		1046	forward 3	TM	N in
		1947	2033	forward 3	TM	N in
37 37	LI:347572.1:2000MAY01	2208	2288	forward 3	TM	N in
37	LI:347572.1:2000MAY01	2412	2477	forward 3	TM	N in
37	LI:347572.1:2000MAY01	2604	2684	forward 3	TM	N in
37	Ll:347572.1:2000MAY01	2739	2795	forward 3	TM	N in
38	LI:817314.1:2000MAY01	460	546	forward 1	TM	
38	LI:817314.1:2000MAY01		1278	forward 1	TM	
38	LI:817314.1:2000MAY01		1386	forward 1	TM	
38	LI:817314.1:2000MAY01	1423	1485	forward 1	TM	
38	LI:817314.1:2000MAY01		1599	forward 1	TM	
38	Ll:817314.1:2000MAY01	1630	1692	forward 1	TM	
38	LI:817314.1:2000MAY01	1756	1842	forward 1	TM	
38	Ll:817314.1:2000MAY01	1930	1992	forward 1	MT	
38	LI:817314.1:2000MAY01		2094	forward 1	TM	
38	LI:817314.1:2000MAY01	2860		forward 1	TM	
38	LI:817314.1:2000MAY01	3127		forward 1	TM	
38	LI:817314.1:2000MAY01	362	448	forward 2	MT	N in
38	LI:817314.1:2000MAY01	3158	3244	forward 2	TM	N in
38	LI:817314.1:2000MAY01	30	95	forward 3	TM	N out
38	Ll:817314.1:2000MAY01	1239	1301	forward 3	TM	N out
38	LI:817314.1:2000MAY01	1785	1865	forward 3	TM	N out
38	Ll:817314.1:2000MAY01	1920	2000	forward 3	TM	N out
38	Ll:817314.1:2000MAY01	3189	3269	forward 3	TM	N out
39	Ll:000290.1:2000MAY01	1003	1065	forward 1	MT	N in
39	LI:000290.1:2000MAY01	1075	1137	forward 1	TM	N in

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39	LI:000290.1:2000MAY01	1195	1248	forward 1	TM	N in
39	LI:000290.1:2000MAY01	767	844	forward 2	TM	
39	LI:000290.1:2000MAY01	882	932	forward 3	TM	N in
40	LI:023518.3:2000MAY01	28	108	forward 1	TM	N out
40.	LI:023518.3:2000MAY01	20	106	forward 2	TM	N in
41	Li:1084246.1:2000MAY01	178	264	forward 1	TM	N out
41	LI:1084246.1:2000MAY01	2686	2760	forward 1	TM	N out
41	LI:1084246.1:2000MAY01	2932	3003	forward 1	TM	N out
41	LI:1084246.1:2000MAY01	3097	3159	forward 1	TM	N out
41	LI:1084246.1:2000MAY01	3184	3246	forward 1	TM	N out
41	LI:1084246.1:2000MAY01	3352	3405	forward 1	TM	N out
41	LI:1084246.1:2000MAY01	3409	3480	forward 1	TM	N out
41	LI:1084246.1:2000MAY01	3526	3609	forward 1	TM	N out
41	LI:1084246.1:2000MAY01	200	253	forward 2	TM	N in
41	LI:1084246.1:2000MAY01	2171	2254			
				forward 2	TM	N in
41	LI:1084246.1:2000MAY01	2654 3065	2734	forward 2	TM	·Nin
41	LI:1084246.1:2000MAY01		3142	forward 2	TM	N in
41	LI:1084246.1:2000MAY01	3284	3358	forward 2	TM	N in
41	LI:1084246.1:2000MAY01	3479	3553	forward 2	TM	N in
41	LI:1084246.1:2000MAY01	582	641	forward 3	TM	N out
41	LI:1084246.1:2000MAY01	2127	2213	forward 3	TM	N out
41	LI:1084246.1:2000MAY01	2457	2543	forward 3	TM	N out
41	LI:1084246.1:2000MAY01	2580	2666	forward 3	TM	N out
41	LI:1084246.1:2000MAY01	2751	2813	forward 3	TM	N out
41	LI:1084246.1:2000MAY01	2826	2888	forward 3	TM	N out
41	LI:1084246.1:2000MAY01	2961	3047	forward 3	TM	N out
41	LI:1084246.1:2000MAY01	3249	3335	forward 3	TM	N out
41	LI:1084246.1:2000MAY01	3429	3515	forward 3	TM	N out
42	LI:1165828.1:2000MAY01	61	147	forward 1	TM	N out
42	LI:1165828.1:2000MAY01	244	312	forward 1	TM	N out
42	LI:1165828.1:2000MAY01	454	510	forward 1	TM	N out
42	LI:1165828.1:2000MAY01	3664	3750	forward 1	TM	N out
42	LI:1165828.1:2000MAY01	3937	4023	forward 1	TM	N out
42	LI:1165828.1:2000MAY01	4600	4653	forward 1	TM	N out
42	LI:1165828.1:2000MAY01	4855	4941	forward 1	TM	N out
42	LI:1165828.1:2000MAY01	5047	5133	forward 1	TM	N out
42	LI:1165828.1:2000MAY01	5227	5298	forward 1	TM	N out
42	LI:1165828.1:2000MAY01	5311	5388	forward 1	TM	N out
42	LI:1165828.1:2000MAY01	5491	5577	forward 1	TM	N out
42	LI:1165828.1:2000MAY01	5800	5871	forward 1	TM	N out
42	LI:1165828.1:2000MAY01	227	301	forward 2	TM	N in
42	LI:1165828.1:2000MAY01	713	775	forward 2	TM	N in
42	LI:1165828.1:2000MAY01	1769	1819	forward 2	TM	N in
42	LI:1165828.1:2000MAY01	2759		forward 2	TM	N in
42	LI:1165828.1:2000MAY01	3869		forward 2	TM	N in
42	LI:1165828.1:2000MAY01	4688		forward 2	TM	N in
42	LI:1165828.1:2000MAY01	5048		forward 2	TM	N in
42	LI:1165828.1:2000MAY01	5531	5617	forward 2	TM	N in
42	LI:1165828.1:2000MAY01	5816		forward 2	TM	N in
42	LI:1165828.1:2000MAY01	39	113	forward 3	TM	N out
42	LI:1165828.1:2000MAY01	906	968	forward 3	TM	N out
42	LI:1165828.1:2000MAY01	1602	1688	forward 3	TM	N out
42	LI:1165828.1:2000MAY01	3471	3557	forward 3	TM	N out
42	LI:1165828.1:2000MAY01	3558	3608	forward 3	TM	N out
42	LI:1165828.1:2000MAY01	4203		forward 3	TM	N out
42	LI:1165828.1:2000MAY01	4749		forward 3	TM	N out
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42	LI:1165828.1:2000MAY01	5625	5690	forward 3	TM	N out
42	LI:1165828.1:2000MAY01	5847	5918	forward 3	TM	N out
43	LI:007302.1:2000MAY01	346	426	forward 1	TM	N in
43	LI:007302.1:2000MAY01	2638	2721	forward 1	TM	N in
43	LI:007302.1:2000MAY01	59	145	forward 2	TM	N out
43	LI:007302.1:2000MAY01	653	718	forward 2	TM	N out
43	LI:007302.1:2000MAY01	1799	1885	forward 2	TM	N out
43	LI:007302.1:2000MAY01	321	407	forward 3	TM	N in
43	LI:007302.1:2000MAY01	480	566	forward 3	TM	N in
43	LI:007302.1:2000MAY01	645	704	forward 3	TM	N in
43	LI:007302.1:2000MAY01	807	890	forward 3	TM	N in
43	LI:007302.1:2000MAY01	1161	1223	forward 3	TM	N in
43	Ll:007302.1:2000MAY01	1236	1298	forward 3	TM	N in
43	LI:007302.1:2000MAY01	1362	1448	forward 3	TM	N in
43	LI:007302.1:2000MAY01	1809	1868	forward 3	TM	N in
43	LI:007302.1:2000MAY01	1998	2084	forward 3	TM	N in
43	LI:007302.1:2000MAY01	2184	2234	forward 3	TM	N in
43	LI:007302.1:2000MAY01	2457	2540	forward 3	TM	N in
43	LI:007302.1:2000MAY01	2595	2681	forward 3	TM	N in
44	LI:236386.4:2000MAY01	3739	3792	forward 1	TM	N out
44	LI:236386.4:2000MAY01	53	118	forward 2	TM	N out
44	LI:236386.4:2000MAY01	218	304	forward 2	TM	N out
44	LI:236386.4:2000MAY01	3755	3823	forward 2	TM	N out
44	LI:236386.4:2000MAY01	2376	2435	forward 3	TM	N out
45	LI:252904.5:2000MAY01	494	550	forward 2	TM	N out
45	LI:252904.5:2000MAY01	300	374	forward 3	TM	N out

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289	564	1501	1591	1800	2266	2344	2619	2500	2310	2658	1950	2210	2658	2247	2402	2663	2764	2658	2577	2549	2667	2658	2658	2667	2766	2658	2760	2658	2658	2658	2658	2621	609
24	ဗ္ဗ	1288	1322	1326	2028	2056	2063	2077	2077	2104	1709	1917	2106	1960	2134	2213	2254	2282	2300	2302	2307	2343	2354	2394	2396	2398	2467	2516	2516	2516	2516	2516	219
3296833H1	492559R1	3903656H1	2554026H1	g1894266	3151953H1	6357422H1	382301T6	2498615F6	2498615H1	492559F1	2684917H1	3898190H1	381716F1	5952437H1	4701147H1	g5435909	7067611H1	g2563607	1889064H1	2400488H1	g817549	g566965	g1894154	6096986	g4291206	g646309	3249908H1	672907H1	672763R6 ·	672763H1	672696H1	672763T6	g1939101
œ	က	ო	က	က	ო	က	က	က	က	က	က	က	က	ო	က	က	က	က	က	က	က	က	က	က	က	က	က	က	က	က	က	က	4
1388	1209	1380	1412	193	662	634	1914	1542	1891	1734	2172	275	594	639	1037	1339	1123	1064	1153	1235	79	1194	1188	244	1347	1192	273	1184	471	1268	1294	1413	1521
1061	1084	1084	1112	<del>-</del> -	125	352	1390	1428	1460	1643	1659	36	84	369	538	749	96/	962	854	854	<del></del>	855	905	7	906	938	=	983	=	1027	1102	1182	1223
u692230	1617090H1	1617090F6	g1157664	6131346H1	6871387H1	g2279352	7039759H1	6481201H1	6929893H1	160750H1	6201684H1	492554H1	6710369H1	g770845	6710369J1	6866894H1	2045879F6	2045879H1	g677645	g570913	2837088H1	g878213	3637810H1	382301R6	3637810F8	5516287H1	382301H1	310657H1	381716R1	054856H1	2676843H1	2865460H1	5983503H1
-		_	_	2	8	8	က	က	က	ဇ	က	က	တ	ဇ	ო	က	က	က	က	က	က	က	<b>ო</b>	က	က	က	က	ო	က	က	က	თ	က
	Stop		959	621	264	242	337	252	316	349	371	419	406	547	651	596	488	089	677	695	299	933	928	955	979	971	1053	1165	1358	1245	1316	1322	1345
	Start		610	_	က	10	<del>-</del>	=	14	107	131	152	181	286	373	376	380	416	580	611	611	611	689	716	716	716	807	899	971	978	1034	1034	1035
	SEQ ID Component	Q	g5813583	6817504J1	g1989978	4292280H1	483000R6	483000H1	q1424329	3255214H1	1450061H1	5388816H1	955673H1	2109273H1	5980116H1	g828864	3072657H1	2949928H1	6016294H1	q1855323	g1623907	g1855498	g1751162	1309114T6	1309114F6	1309114H1	3637614H1	7065033H1	6817504H1	6013754H1	a573231	a709283	g767017
•	SEO ID	Ö		_	_																												

TABLE 4 (cont.	$\overline{}$
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13	746910T6	1913	2371	4	g4897951	44	477	15	g1921208	645	982
13	6844175H1	1941	2375	14	609028H1	27	178	5	6523810H1	629	1052
13	2568562H1	1989	2222	4	g2782816	15	417	5	3499282H1	423	902
13	g4393425	1996	2415	14	g4326525	<del>-</del>	141	15	5852917H1	661	921
13	g4109519	2006	2375	4	g2525795	28	236	15	2247228H1	692	959
13	g2694947	2036	2375	15	g6450570	1077	1426	15	g851799	704	1030
33	g2703845	2040	2375	15	g6473965	26	472	15	4946358H1	711	972
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13	g3278030	2045	2423	15	g2898932	121	456	5	6345162H1	792	1031
5	4705947H1	2104	2256	15	526619H1	129	370	15	3436737H1	794	1029
5	g714831	2110	2411	15	g2942591	134	271	15	g2264229	456	815
13	750787H1	2121	2365	15	2360586H1	145	399	15	3496822H1	430	203
13	667235H1	2126	2370	15	2211028H1	228	438	15	6321740H1	802	1031
5	g561290	2150	2375	15	987239R1	305	763	15	2112334H1	820	1080
5	g518739	2157	2375	15	987239H1	305	478	5	1007012H1	470	<b>1</b> 92
13	g3230679	2187	2375	15	1436565F1	354	824	15	2112334R6	820	1167
<u>ლ</u>	g717890	2318	2390	15	7161757H1	_	521	15	3215530H1	491	714
14	4145560H1	_	337	15	g4372435	23	212	15	3144904H1	873	1217
4	7182979H1	-	537	15	g5451540	23	516	15	g4073140	965	1444
4	g4929686	<b>,</b> -	1581	15	g3884494	40	407	15	g4523268	920	1426
14	g1881193	113	359	15	g5545276	40	499	15	g5673767	972	1444
4	798770H1	506	449	15	2269559H1	44	305	15	2836020H1	496	741
4	g1198695	214	498	15	2269559R6	44	350	15	960106H1	971	1049
4	g1637735	380	642	15	g5152652	62	224	15	962045H1	971	1248
4	g2204679	39	511	15	3222733H1	98	303	15	5109444H1	498	723
4	5540595H1	<del>-</del>	195	15	1664718F6	91	349	15	g2070246	973	1335
4	g1970769	-	345	15	1664718H1	91	352	15	g2206523	973	1266
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4	g1970777	_	223	15	2520441H1	360	641	15	g5449171	626	1439
4	g815792	80	284	₹ <u></u>	3460138H1	393	644	15	3733518H1	980	1275
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	3097	3098	3101	3101	3103	3109	3111	3117	3132	3132	3132	3133	3136	3138	3143	3150	3150	3150	3151	3151	3151	3151	3153	3163	3163	3170	3330	3332	3341	3349	3357	3385	3401	3487	
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88	28	<b>58</b>	<b>58</b>	88	<b>58</b>	<b>78</b>	<b>58</b>	28	<b>5</b> 8	<b>58</b>	28	<b>5</b> 8	<b>5</b> 8	28	<b>5</b> 8	<b>58</b>	<b>58</b>	<b>58</b>	28	58	28	28	<b>58</b>	<b>58</b>	<b>58</b>	28	28	28	<b>58</b>	<b>58</b>	58	28	28

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g4330537	2837088H1	382301H1	382301R6	381716R1	6853095H1	3296833H1	492559R1	492554H1	6710369H1	g770845	671036931	6866894H1	2045879F6	2045879H1	q677645	g570913	g878213	3637810H1	3637810F8	5516287H1	310657H1	054856H1	2676843H1	2865460H1	5983503F8	5983503H1	6540006H1	3903656H1	2554026H1	a1894266	7039759H1	6481201H1
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TABLE 4 (cont.)		g1382788 2	9	g1501595 2	Ŧ		m		_	Ŧ	g779790 1	· 두	4733091H1 1	2614356H1 1	2614355H1 1	1340369F6 1	1340369H1 1	70920240V1 1	757294H1 1	2658667H1 1	2771444H1 1	1312886F6 1	_	2308711H1 1	3519383H1 1	2306567H1 1	1304465H1 1	5172484H1 1	4172237H1 1	2877775H1 1	869079H1 1	3939024H1 1	71273416V1 1	1420994H1 1
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1427	1383	1538	1427	1389	1303	1429	1448	1426	1544	1631	1418	1464	1726	1742	1599	1224	1592	1729	1718	1693	1182	1260	1186	474	326	2036	581	1086	2662	2207	2612	2705	2673
761	784	792	804	854	856	862	889	938	963	1005	1009	1068	1071	1081	1085	1109	1146	1155	1155	1155	568	564	277	5	<b>-</b>	1465	-	510	2066	2069	2138	2151	2151
70556961V1	<b>70557</b> 092V1	70554523V1	70557219V1	70555075V1	70555282V1	70554784V1	6785373H1	70556389V1	70556118V1	70557489V1	70554717V1	6784929H1	6828695J1	7055600011	6934607H1	70449057V1	71303301V1	5811393F6	71156205V1	71156521V1	70554574V1	70556236V1	70554808V1	6788638H1	6787884H1	71303881V1	6788583H1	6788770H1	70554811V1	4515767H1	71303748V1	70328165D1	70326303D1
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;	2055	2660	2415	2570	1823	1695	1825	45	02	2775	<b>o</b>	29	83	<b>o</b>	68	1406	42	83	20	29	22	29	15	92	61	92	43	43	43	46	26	52	46	35
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	2693	2676	2498	3013	2708	2708	2798	2791	2762	3321	3767	3454	3806	3594	3666	3770	3511	3806	3654	3806	3806	3604	3572	3742	3806	3806	3787	3802	3790	3781	3288	3340	3261	3458
	2331	2331	2331	2430	2452	2479	2572	222	2572	3085	3100	3205	3205	3222	1251	3300	3307	3324	3381	1394	3395	3404	3404	3447	3504	3516	3534	3556	3606	3701	3100	3105	3037	3085
	_		g1482703 2	6549638H1 2	70300848D1 2	70300835D1 2	415443H1 2	419855H1 2	416163H1 2	1739793H1	1739793T6 3	4422806H1 3	415443F1 3	70300638D1 3	70300351D1 3	1595527T6 3	1595527H1 3	415986F1 3	4879243H1 3	g6139643 3		<u>-</u>	2287181R6 3	g1162076 3			_	70300150D1 3					2402302H1 3	1739793R6 3
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4601	3476	4795	4864	4882	4884	3740	4787	4788	_	464	466	4886	4886	4886	4886	4890	4890	4890	4890	4890	4892	4893	4894	4905	4922	4928	4931	4951	4953	4954	4965	4971
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3267	3287	3333	3333	3333	3333	3333	3333	3341	3355	3355	3363	3396	3391	3809	5564	5584	4346	4360	4367	4376	4385	4388	4416	4434	4477	4513	4513	4544	4577	4577	4584	4590
g823731 7044511H1	5919091H1	71188683V1	71191815V1	71191533V1	71191734V1	1600316F6	1600316H1	70867333V1	g839823	g824451	71190867V1	70870265V1	2013807H1	70866888V1	3673862H1	2499983T6	g815044	70869526V1	2499983H1	70867729V1	5386383H1	70868265V1	6274578H1	71190615V1	70866931V1	71189990V1	71190387V1	g672203	1269521F6	1269521H1	71230051V1	g670126
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4079	4094	4111	4165	4167	4173	4176	4177	4184	4190	4190	4197	4197	4218	4238	4253	4297	4303	467	725	725	1334	1334	1387	2401	2815	2815	2815	2973	3182	3181	3257	3256
g612859 71189238V1	g570718	71188405V1	g2805702	g3694501	71189379V1	g6144708	g2323168	g819401	70868193V1	g766671	g1516806	g1525425	g830693	71188787V1	4785755H1	70866811V1	g1614228	g3229742	g5457022	g5456921	g4683485	g5765573	g3075910	7190218H2	71229788V1	5014904F6	5014904H1	71229920V1	71228807V1	6884462H1	70868094V1	70869027V1
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3626 3638 3631 3653 3655 3679 3685 3725 3725 3725 4615 4615 4615 4615 4615	4615 4615 3394 3421 3462 3809 3828 3840
551V1 19V1 554V1 695V1 66V1 664V1 664V1 664V1 662V1 99H1 11 11 11 11 11 11 11 11 11 11 11 11 1	2H1 49V1 73V1 57V1 12V1 24V1 10V1 50V1 4H2
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TABLE 4 (	cont.
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3352 3352 1131 1153 1277 1042 3218 3676 3684 3684 3684 3693 3693 3791 3740 3740	3816 3816 3816 3824 3527 3527 3536 3546 3546 3565 3565 3565
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6559394H1 3382113H1 70606021V1 70879980V1 2661806H1 70879113V1 96476309 2627073H1 2627073H1 2627073H1 2627315H1 3901711H1 70881572V1 6969302U1 70881572V1 5763849H1 7256511H1	70880211V1 7088271V1 70881365V1 7008393D1 70012299D1 7001229D1 7001229D1 7001229D1 7001229D1 7001229D1 7001229D1 7001229D1 7001229D1 7001229D1 70010847D1 70880257V1 70880257V1 70888761V1
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2702 2733 2762 1415 1146 2940 2945 2910 2923 2714 2897 2663 3835 3637 3736 3736	3589 3749 3672 3797 3701 2043 2049 1942 1752 1752 1752 1941 1990
2417 1 1 953 953 2634 2634 2634 2634 2634 2634 2634 263	3312 3442 3415 3420 3420 1419 1480 1492 1494 1571 1608 1616 1622 1622 1622
6377332H1 4947810H1 95006247 5540505F6 5540505H1 92875734 93735348 5118201T6 2749265F6 2749265F1 2749265T1 1452312F1 70007188D1 9898311 1452312F1 1452312F1	259900/HI 6325947HI 840648HI 70012088DI 5852153HI 70604010VI 6952285HI 4458494F6 70608095VI 4458494HI 7255931H2 6909665JI 6909665JI 6909665JI 6909665JI 7255931H2 6909665JI 6909665JI
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	3602	3605	3606	3609	3609	3609	3621	3624	3660	3673	3367	3415	3415	3400	3404	3218	1033	1034	3442	3442	3129	3137	3145	3156	1080	1108	2904	2904	2910	2909	2910	2922	2923	2940
	g6451467	g1521304	g4534027	5790863H1	5789451H1	5787849H1	g5528373	g1516463	g5912966	344685H1	2623608H1	840648R1	4333836H1	70881547V1	70886619V1	2414749F6	70605048V1	7267489H1	6346421H1	6317150H1	4897563H1	5379052H1	3406784H1	70008878D1	70608052V1	g3888759	2857322H1	70881851V1	792748R1	792748H1	793130H1	7159471H1	70880131V1	1541872H1

## TABLE 4 (cont.)

3698 45 1524230H1 43 3454 45 3384786H1 92	45 6055559H1	45 6055841H1	3415 45 4509676H1 259	3382 45 3081417H1 405	3351 45 2952165H1 422	3637 45 70874349V1 542	3364	3440	167	705	794	610	209	635	635	1146	1414	1795	1922	1601	1770	1982		235		232	4	296	1	Ε.	
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g1517127 2414483H1	-		70003405D1	70007838D1		70012577D1	1320150H1	70008556D1	4181419H1	6779195J1	113399R6	4507995F6	4507995H1	6831490H1	6831490J1	70604944V1	70607511V1	6454789H1	70603538V1	684735H1	70607606V1	70603837V1	70006129D1	3386984H1	3087717H1	4832592H1	3750644H1	3350574H1	3150464H1	3381160H1	

TABLE 5

		TABLE 5
SEQ ID NO:	Template ID	Tissue Distribution
-	LG:977683.1:2000FEB18	Nervous System - 21%, Skin - 19%, Embryonic Structures - 11%
7	LG:893050.1:2000FEB18	Digestive System - 40%, Hemic and Immune System - 40%, Nervous System - 20%
က	LG:980153.1:2000FEB18	Nervous System - 16%, Urinary Tract - 12%, Skin - 12%
4	LG:350398.1:2000FEB18	Digestive System - 50%, Hemic and Immune System - 50%
2	LG:475551.1:2000FEB18	Skin - 35%, Hemic and Immune System - 19%, Digestive System - 11%
9	LG:481407.2:2000FEB18	widely distributed
	LI:443580.1:2000FEB01	Unclassified/Mixed - 60%, Connective Tissue - 17%, Endocrine System - 13%
89	LI:803015.1:2000FEB01	Urinary Tract - 63%, Respiratory System - 38%
Ø	LG:027410.3:2000MAY19	Respiratory System - 100%
10	LG:171377.1:2000MAY19	Unclassified/Mixed - 74%, Female Genitalia - 13%, Cardiovascular System - 10%
=	LG:352559.1:2000MAY19	Unclassified/Mixed - 71%, Digestive System - 29%
		Stomatognathic System - 39%, Musculoskeletal System - 28%, Cardiovascular
12	LG:247384.1:2000MAY19	System - 19%
13	LG:403872.1:2000MAY19	Nervous System - 40%, Embryonic Structures - 23%, Urinary Tract - 14%
14	LG:1135213.1:2000MAY19	Embryonic Structures - 24%, Cardiovascular System - 20%, Unclassified/Mixed - 13%
15	LG:474284.2:2000MAY19	Unclassified/Mixed - 14%
16	LG:342147.1:2000MAY19	Pancreas - 21%, Male Genitalia - 19%, Female Genitalia - 17%, Urinary Tract - 17%
17	LG:1097300.1:2000MAY19	Endocrine System - 25%, Skin - 18%, Unclassified/Mixed - 13%
18	LG:444850.9:2000MAY19	Digestive System - 28%, Connective Tissue - 20%, Exocrine Glands - 10%
19	LG:402231.6:2000MAY19	Endocrine System - 23%, Hemic and Immune System - 23%, Digestive System - 18%
50	LG:1076157.1:2000MAY19	Embryonic Structures - 50%, Endocrine System - 28%, Respiratory System - 17%
21	LG:1083142.1:2000MAY19	Germ Cells - 84%
22	LG:1083264.1:2000MAY19	Liver - 52%, Connective Tissue - 33%
23	LG:350793.2:2000MAY19	Sense Organs - 25%, Connective Tissue - 14%
24	LG:408751.3:2000MAY19	Nervous System - 39%, Sense Organs - 39%
25	LI:336120.1:2000MAY01	Nervous System - 24%, Respiratory System - 22%, Endocrine System - 18%
26	LI:234104.2:2000MAY01	Female Genitalia - 21%, Unclassified/Mixed - 17%, Nervous System - 12%
27	LI:450887.1:2000MAY01	Nervous System - 100%
28	LI:119992.3:2000MAY01	Embryonic Structures - 10%
59	LI:197241.2:2000MAY01	Connective Tissue - 26%, Endocrine System - 12%
30	LI:406860.20:2000MAY01	Digestive System - 100%
3	LI:142384.1:2000MAY01	Connective Tissue - 44%, Germ Cells - 34%
35	LI:895427.1:2000MAY01	Cardiovascular System - 20%, Urinary Tract - 14%, Skin - 13%
33	LI:757439.1:2000MAY01	Digestive System - 18%, Embryonic Structures - 13%, Sense Organs - 12%

0MAY01 Cardiovascular System - 59%, Exocrine Glands - 25% MAY01 Pancreas - 63%					Urinary Tract - 50%, Musculoskeletal System - 27%, Hemic and Immune System - 23%		0MAY01 Sense Organs - 72%		Connective Tissue - 29%, Respiratory System - 21%, Hemic and Immune System -		MAY01 Skin - 30%, Female Genitalia - 11%	MAY01 Exocrine Glands - 20%, Nervous System - 16%, Endocrine System - 13%
LI:1144066.1:2000MAY01 LI:243660.4:2000MAY01	LI:334386.1:2000MAY01	LI:347572.1:2000MAY01	LI:817314.1:2000MAY01	LI:000290.1:2000MAY01		LI:023518.3:2000MAY01	LI:1084246.1:2000MAY01	LI:1165828.1:2000MAY01		LI:007302.1:2000MAY01	LI:236386.4:2000MAY01	LI:252904.5:2000MAY01
34 35	36	37	38	33		40	41	42		43	44	45

TABLE

SEQ ID	Frame	Length	Start	Stop	GI Number	Probability	Annotation
NO:						score	
46	3	263	27	815	g10764778	1e-131	phosphoinositol 3-phosphate-binding protein~2 [Homo sapiens]
					~10045840	10-58	
					910043040	26-38	
į		27.2	0.5	023	30000059	19-81	nrotein [Homo
7 5	- <del>-</del>	/17	2	200	96653538	16-91 6e-77	ical protein [H
					g4803678	7e-29	ankyrin (brank-2) [Homo sapiens]
48	1	716	613	2760	g7243215	0.0	KIAA1417 protein [Homo sapiens]
	1				g7263990	0.0	dJ93K22.1 (novel protein (contains DKF2P564B116)) [Homo sapiens]
					g7302944	5e-57	CG8060 gene product [Drosophila melanogaster]
49	3	107	09	380			
50	3	645	3	1937	94826478	0.0	dJ37E16.2 (SH3-domain binding protein 1) [Homo
					g861029	0.0	- 1
					g7018521	0.0	protein [
51	3	177	93	623	g6119546	1e-45	hypothetical protein; 114721-113936
5	)	· · ·					
					g6522593	3e-10	putative RNA binding protein (Arabidopsis thaliana]
					r950424	4e-10	splicing factor, arginine/serine-rich 7 [Homo
					יו פור פור	)	
52	-	217	79	729	g4589566	3e-34	
l )	l 				g3970712	3e-26.	zinc finger protein 10 [Homo sapiens]
					g7630121	8e-25	zinc finger protein 92 [Mus musculus]
53	3	151	3	455	g5262560	2e-35	hypothetical protein [Homo sapiens]
}	)				g10434856	1e-29	unnamed protein product [Homo sapiens]
					g930123	9e-27	zinc finger protein (583 AA) [Homo sapiens]
54	3	193	3	581	g10438267	1e-74	unnamed protein product (Homo sapiens)
s )	)				g7290756	8e-16	
					g5705877	8e-10	
դ አ	3	282	<u>س</u>	848	g3077703	1e-111	mitsugumin29 [Oryctolagus cuniculus]
}	1				g3461888	1e-108	[Mus
					g3761107	1e-108	mitsugumin29 [Mus musculus]

TABLE 6 (cont.)

SEO ID	Frame	Length	Start	Stop	GI Number	Probability	Annotation
•		n n		4		score	
56	2	211	2	634	g7243243	2e-44	KIAA1431 protein [Homo sapiens]
					g4567179	2e-43	BC37295_1 [Homo sapiens]
					g3445181	1e-41	R31665_2 [Homo sapiens]
57	2	366	83	1180	g9945010	1e-120	RING-finger protein MURF [Mus musculus]
					g9929937	5e-92	hypothetical protein [Macaca fascicularis]
					g10439844	1e-36	unnamed protein product [Homo sapiens]
58	3	326	354	1331	g7020303	0.0	unnamed protein product [Homo sapiens]
					g10434892	3e-79	unnamed protein product [Homo sapiens]
					g6683707	2e-31	KIAA0455 protein [Homo sapiens]
59	1	156	70	537	g6692607	2e-69	MGA protein [Mus musculus]
					g5931585	9e-47	T-box family member; T-box domain [Cynops
							pyrrhogaster]
					g4049463	3e-16	transcription factor TBX6 (Homo sapiens)
09	2	262	239	1024	g1488047	7e-12	RING finger protein [Xenopus laevis]
					g3916727	1e-11	estrogen-responsive B box protein (Homo
					g401763	1e-11	ataxia-telangiectasia group D-associated
							protein (Homo sapiens)
61	3	132	138	533			
62	2	167	2	502	g2078531	2e-71	Mlark [Mus musculus]
<u> </u>					g2078529	2e-70	Hlark [Homo sapiens]
					g1149523	8e-57	
63	1	570	160	1869	g183002	0.0	guanylate binding protein isoform I [Homo
					g829177	0.0	guanylate binding protein isoform II (Homo
					g7023332	0.0	unnamed protein product [Homo sapiens]
64	3	168	3	905	g7020737	2e-89	ω,
					g8920240	2e-89	hypothetical protein,
							PRAJAI
					g2979531	2e-51	R33683_3 [Homo sapiens]

TABLE 6 (cont.)

SEQ ID	Frame	Length	Start	Stop	GI Number	Probability	Annotation
NO:				,		score	
65	3	246	57	794	g5262560	3e-65	hypothetical protein [Homo sapiens]
					g10434856	4e-64	ein product (Ho
					g930123	7e-56	zinc finger protein (583 AA) [Homo sapiens]
99	3	120	51	410	g4589566	2e-23	KIAA0961 protein [Homo sapiens]
					g456269	7e-22	zinc finger protein 30 (Mus musculus
							domesticus]
					g5080758	2e-20	BC331191_1 [Homo sapiens]
67	2	122	329	694	g10047297	7e-26	KIAA1611 protein [Homo sapiens]
		-			g8163824	2e-19	krueppel-like zinc finger protein HZF2 [Homo
					93329372	61-99	DNA-binding protein [Homo sapiens]
89	3	428	132	1415	g6094684	0.0	similar to Kelch proteins; similar to
							BAA77027 (PID:g4650844) [Homo sapiens]
					g7242973	0.0	KIAA1309 protein [Homo sapiens]
					g7243089	0.0	KIAA1354 protein [Homo sapiens]
69	2	307	2	922	98671168	1e-135	hypothetical protein [Homo sapiens]
				_	98886025	1e-135	collapsin response mediator protein-5 [Homo
							sapiens]
					g8671360	1e-131	Ulip-like protein [Rattus norvegicus]
70	1	198	928	1449	g1864085	1e-103	glypican-5 [Homo sapiens]
					g3015542	1e-103	glypican-5 [Homo sapiens]
					g205800	7e-38	intestinal protein OCI-5 (Rattus norvegicus)
71		227	511	1191	g1155088	1e-06	zyxin (Homo sapiens)
					g1545954	1e-06	zyxin [Homo sapiens]
					g576623	2e-06	ESP-2 [Homo sapiens]
72	3	122	3	368	g7629994	4e-41	60S RIBOSOMAL PROTEIN L36 homolog
							[Arabidopsis thaliana]
					g3236242	5e-40	60S ribosomal protein L36 (Arabidopsis
							thaliana]
				<u> </u>	g11908070	5e-40	60S ribosomal protein-like protein
							[Arabidopsis thaliana]

TABLE 6 (cont.)

SEQ ID	Frame	Length	Start	Stop	GI Number	Probability	Annotation
 0	!					score	
73	2	209	200	1126	g10435614	1e-113	protein product
					g7243089	1e-113	KIAA1354 protein [Homo sapiens]
					g7242973	1e-107	KIAA1309 protein [Homo sapiens]
74		312	961	1896	g7243215	1e-157	KIAA1417 protein [Homo sapiens]
•	l 				q7263990	1e-157	dJ93K22.1 (novel protein (contains
							DKFZP564B116)) [Homo sapiens]
					g7302944	3e-17	CG8060 gene product [Drosophila melanogaster]
75	<u>س</u>	190	3	572	g10435919	69-e9	unnamed protein product [Homo sapiens]
)	1				g3327128	3e-33	KIAA0657 protein [Homo sapiens]
					g10436504	4e-09	unnamed protein product [Homo sapiens]
76	9	295	3	887	g10436290	1e-105	
•	1				g10436002	66-99	unnamed protein product [Homo sapiens]
					g8489831	2e-27	ubiquitin-conjugating BIR-domain enzyme
							APOLLON [Homo sapiens]
77	2	288	374	1237	g3184264	5e-94	F02569_2 [Homo sapiens]
	l				g10435546	5e-84	unnamed protein product [Homo sapiens]
					g6653742	4e-54	7h3 protein [Homo sapiens]
78	-	294	97	978	g7670362	1e-106	unnamed protein product [Mus musculus]
<b>)</b>	1	1			g6175860	4e-15	g1-related zinc finger protein [Mus musculus]
					g6330555	1e-13	KIAA1214 protein [Homo sapiens]
79	<u>m</u>	196	3	590	g3513300	3e-65	F16601_1, partial CDS [Homo sapiens]
<b>1</b>					g3882281	3e-50	- 1
					g10567164	4e-50	gene amplified in squamous cell carcinoma-1
	_						[Homo sapiens]
80	<u>س</u>	745	285	2519	g2224553	0.0	KIAA0306 [Homo sapiens]
	,				g4210501	0.0	ł
					g10728201	3e-20	CG2779 gene product [Drosophila melanogaster]
81	3	256	507	1274	g6330617	1e-132	KIAA1223 protein [Homo sapiens]
l i	·				g7301689	2e-72	CG10011 gene product [Drosophila
		-					
					g4803678	2e-33	ankyrin (brank-2) [Homo sapiens]

TABLE 6 (cont.)

SEQ ID	Frame	Length	Start	Stop	GI Number	Probability	Annotation
NO:						score	
82	1	235	841	1545	g9802433	2e-76	ACE-related carboxypeptidase ACE2 [Homo sapiens]
					g5817160	2e-76	hypothetical protein [Homo sapiens]
					g11876766	2e-76	unnamed protein product [Homo sapiens]
83	1	219	229	2079	96665594	0.0	trp-related protein 4 truncated variant delta
							[Homo sapiens]
					g6665592	0.0	trp-related protein 4 truncated variant beta
							[Homo sapiens]
					g6665590	0.0	trp-related protein 4 [Homo sapiens]
84	3	293	735	1613	g7242977	1e-143	KIAA1311 protein [Homo sapiens]
					g912755	2e-15	B0336.3 gene product [Caenorhabditis elegans]
					g7298595	8e-12	CG10084 gene product [Drosophila
							melanogaster]
85	3	276	30	857	g3955100	2e-74	vacuolar adenosine triphosphatase subunit D
							[Mus musculus]
					g1226235	2e-74	Ac39/physophilin [Mus musculus]
					g736727	2e-74	32 kd accessory protein [Bos taurus]
98	3	355	1392	2456	g5457043	0.0	protocadherin beta 4 [Homo sapiens]
					g11142065	0.0	protocadherin beta 9 [Homo sapiens]
					g8926617	0.0	protocadherin 3H (Homo sapiens)
87	2	745	716	2950	g5457023	0.0	protocadherin alpha 9 short form protein
							[Homo sapiens]
		- <b></b>			g3540157	0.0	KIAA0345-like 5 [Homo sapiens]
					g2224631	0.0	KIAA0345 [Homo sapiens]
88	2	781	50	2392	g5006248	0.0	TLR6 [Homo sapiens]
					g11596326	0.0	toll-like receptor 6 [Mus musculus]
					g5006250	0.0	TLR6 (Mus musculus)
89	2	293	1313	2191	g6164628	2e-27	SH3 and PX domain-containing protein SH3PX1
							[Homo sapiens]
					g5327052	2e-27	dJ403L10.1 (SNX9 (Sorting Nexin 9)) (Homo
							sapiens)
					g4689258	2e-27	sorting nexin 9 (Homo sapiens)

TABLE 6 (cont.)

	t [Homo sapiens]	sapiens)	in similar to	Canal protein, KEL)	of other types of	s]
Annotation	unnamed protein product [Homo sapiens	KIAA0795 protein [Homo sapiens]	dA22D12.1 (novel protein similar to	Drosophila Kelch (Ring Canal protein, KEL)	and a heterogenous set of other types of	proteins) [Homo sapiens]
Probability Annotation score	1e-62	4e-15	46-14			
GI Number	g7022971	93882311	g4539520			
Stop	936					
Start	214					
Frame Length Start	241					
Frame	1					
SEQ ID NO:	90					

## Table,

Program	Description	Reference	Parameter Threshold
ABI FACTURA	A program that removes vector sequences and masks ambiguous bases in nucleic acid sequences.	Applied Biosystems, Foster City, CA.	
ABIPARACEL FDF	A Fast Data Finder useful in comparing and annotating amino acid or nucleic acid sequences.	Applied Biosystems, Foster City, CA; Paracel Inc., Pasadena, CA.	. Mismatch <50%
ABI AutoAssembler	A program that assembles nucleic acid sequences.	Applied Biosystems, Foster City, CA.	
BLAST	A Basic Local Alignment Search Tool useful in sequence similarity search for amino acid and nucleic acid sequences. BLAST includes five functions: blastp, blastn, blastx, tblastn, and tblastx.	Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410; Altschul, S.F. et al. (1997) Nucleic Acids Res. 25:3389-3402.	ESTs: Probability value= 1.0E-8 or less Full Length sequences: Probability value= 1.0E-10 or less
107	A Pearson and Lipman algorithm that searches for similarity between a query sequence and a group of sequences of the same type. FASTA comprises as least five functions: fasta, tfasta, tfastx, and ssearch.	Pearson, W.R. and D.J. Lipman (1988) Proc. Natl. Acad Sci. USA 85:2444-2448; Pearson, W.R. (1990) Methods Enzymol. 183:63-98; and Smith, T.F. and M.S. Waterman (1981) Adv. Appl. Math. 2:482-489.	ESTs: fasta E value=1.06B-6 Assembled ESTs: fasta Identity= 95% or greater and Match length=200 bases or greater; fastx E value=1.0B-8 or less Full Length sequences: fastx score=100 or greater
BLIMPS	A BLocks IMProved Searcher that matches a sequence against those in BLOCKS, PRINTS, DOMO, PRODOM, and PFAM databases to search for gene families, sequence homology, and structural fingerprint regions.	Henikoff, S. and J.G. Henikoff (1991) Nucleic Acids Res. 19:6565-6572; Henikoff, J.G. and S. Henikoff (1996) Methods Enzymol. 266:88-105; and Attwood, T.K. et al. (1997) J. Chem. Inf. Comput. Sci. 37:417-424.	Probability value= 1.0E-3 or less
HMMER	An algorithm for searching a query sequence against hidden Markov model (HMM)-based databases of protein family consensus sequences, such as PFAM.	Krogh, A. et al. (1994) J. Mol. Biol., 235:1501-1531; Sonnhammer, E.L.L. et al. (1988) Nucleic Acids Res. 26:320-322; Durbin, R. et al. (1998) Our World View, in a Nutshell, Cambridge Univ. Press, pp. 1-350.	PFAM hits: Probability value=1.0E-3 or less Signal peptide hits: Score=0 or greater

## Table 7 (cont.)

	Program	Description	Reference	Parameter Threshold
	ProfileScan	An algorithm that searches for structural and sequence motifs in protein sequences that match sequence patterns defined in Prosite.	Gribskov, M. et al. (1988) CABIOS 4:61-66; Gribskov, M. et al. (1989) Methods Enzymol. 183:146-159; Bairoch, A. et al. (1997) Nucleic Acids Res. 25:217-221.	Normalized quality score SCG-specified "HIGH" value for that particular Prosite motif. Generally, score=1.4-2.1.
	Phred	A base-calling algorithm that examines automated sequencer traces with high sensitivity and probability.	Ewing, B. et al. (1998) Genome Res. 8:175-185; Ewing, B. and P. Green (1998) Genome Res. 8:186-194.	
	Phrap	A Phils Revised Assembly Program including SWAT and CrossMatch, programs based on efficient implementation of the Smith-Waterman algorithm, useful in searching sequence homology and assembling DNA sequences.	Smith, T.F. and M.S. Waterman (1981) Adv. Appl. Math. 2:482-489; Smith, T.F. and M.S. Waterman (1981) J. Mol. Biol. 147:195-197; and Green, P., University of Washington, Seattle, WA.	Score= 120 or greater; Match length= 56 or greater
10	Consed	A graphical tool for viewing and editing Phrap assemblies.	Gordon, D. et al. (1998) Genome Res. 8:195-202.	
8	SPScan	A weight matrix analysis program that scans protein sequences for the presence of secretory signal peptides.	Nielson, H. et al. (1997) Protein Engineering 10:1- 6; Claverie, J.M. and S. Audic (1997) CABIOS 12:431-439.	Score=3.5 or greater
=	ТМАР	A program that uses weight matrices to delineate transmembrane segments on protein sequences and determine orientation.	Persson, B. and P. Argos (1994) J. Mol. Biol. 237:182-192; Persson, B. and P. Argos (1996) Protein Sci. 5:363-371.	
r L	ТМНММЕК	A program that uses a hidden Markov model (HMM) to delineate transmembrane segments on protein sequences and determine orientation.	Sonnhammer, E.L. et al. (1998) Proc. Sixth Intl. Conf. on Intelligent Systems for Mol. Biol., Glasgow et al., eds., The Am. Assoc. for Artificial Intelligence Press, Menlo Park, CA, pp. 175-182.	
4	Motifs	A program that searches amino acid sequences for patterns that matched those defined in Prosite.	Bairoch, A. et al. (1997) Nucleic Acids Res. 25:217-221; Wisconsin Package Program Manual, version 9, page M51-59, Genetics Computer Group, Madison, WI.	

## CLAIMS

## What is claimed is:

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An isolated polynucleotide comprising a polynucleotide sequence selected from the group
 consisting of:

- a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-45,
- b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-45,
  - c) a polynucleotide sequence complementary to a),
  - d) a polynucleotide sequence complementary to b), and
  - e) an RNA equivalent of a) through d).
- 2. An isolated polynucleotide of claim 1, comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-45.

3. An isolated polynucleotide comprising at least 60 contiguous nucleotides of a polynucleotide of claim 1.

- 4. A composition for the detection of expression of disease detection and treatment molecule polynucleotides comprising at least one of the polynucleotides of claim 1 and a detectable label.
  - 5. A method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide of claim 1, the method comprising:
- a) amplifying said target polynucleotide or fragment thereof using polymerase chain reaction
   amplification, and
  - b) detecting the presence or absence of said amplified target polynucleotide or fragment thereof, and, optionally, if present, the amount thereof.
- 6. A method for detecting a target polynucleotide in a sample, said target polynucleotide comprising a sequence of a polynucleotide of claim 1, the method comprising:
  - a) hybridizing the sample with a probe comprising at least 20 contiguous nucleotides comprising a sequence complementary to said target polynucleotide in the sample, and which probe specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization complex is formed between said probe and said target polynucleotide or fragments thereof, and

b) detecting the presence or absence of said hybridization complex, and, optionally, if present, the amount thereof.

- 7. A method of claim 5, wherein the probe comprises at least 30 contiguous nucleotides.
- 8. A method of claim'5, wherein the probe comprises at least 60 contiguous nucleotides.
- 9. A recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide of claim 1.
  - 10. A cell transformed with a recombinant polynucleotide of claim 9.

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- 11. A transgenic organism comprising a recombinant polynucleotide of claim 9.
- 12. A method for producing a disease detection and treatment molecule polypeptide, the method comprising:
  - a) culturing a cell under conditions suitable for expression of the disease detection and treatment molecule polypeptide, wherein said cell is transformed with a recombinant polynucleotide of claim 9, and
    - b) recovering the disease detection and treatment molecule polypeptide so expressed.
  - 13. A purified disease detection and treatment molecule polypeptide (MDDT) encoded by at least one of the polynucleotides of claim 2.
- 25 14. An isolated antibody which specifically binds to a disease detection and treatment molecule polypeptide of claim 13.
  - 15. A method of identifying a test compound which specifically binds to the disease detection and treatment molecule polypeptide of claim 13, the method comprising the steps of:
    - a) providing a test compound;
  - b) combining the disease detection and treatment molecule polypeptide with the test compound for a sufficient time and under suitable conditions for binding; and

c) detecting binding of the disease detection and treatment molecule polypeptide to the test compound, thereby identifying the test compound which specifically binds the disease detection and treatment molecule polypeptide.

- 16. A microarray wherein at least one element of the microarray is a polynucleotide of claim 3.
- 17. A method for generating a transcript image of a sample which contains polynucleotides, the method comprising the steps of:
  - a) labeling the polynucleotides of the sample,

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- b) contacting the elements of the microarray of claim 16 with the labeled polynucleotides of the sample under conditions suitable for the formation of a hybridization complex, and
  - c) quantifying the expression of the polynucleotides in the sample.
- 18. A method for screening a compound for effectiveness in altering expression of a target polynucleotide, wherein said target polynucleotide comprises a polynucleotide sequence of claim 1, the method comprising:
- a) exposing a sample comprising the target polynucleotide to a compound, under conditions suitable for the expression of the target polynucleotide,
  - b) detecting altered expression of the target polynucleotide, and
- c) comparing the expression of the target polynucleotide in the presence of varying amounts of the compound and in the absence of the compound.
  - 19. A method for assessing toxicity of a test compound, said method comprising:
  - a) treating a biological sample containing nucleic acids with the test compound;
- b) hybridizing the nucleic acids of the treated biological sample with a probe comprising at least 20 contiguous nucleotides of a polynucleotide of claim 1 under conditions whereby a specific hybridization complex is formed between said probe and a target polynucleotide in the biological sample, said target polynucleotide comprising a polynucleotide sequence of a polynucleotide of claim 1 or fragment thereof;
  - c) quantifying the amount of hybridization complex; and
- d) comparing the amount of hybridization complex in the treated biological sample with the amount of hybridization complex in an untreated biological sample, wherein a difference in the amount of hybridization complex in the treated biological sample is indicative of toxicity of the test compound.

20. An array comprising different nucleotide molecules affixed in distinct physical locations on a solid substrate, wherein at least one of said nucleotide molecules comprises a first oligonucleotide or polynucleotide sequence specifically hybridizable with at least 30 contiguous nucleotides of a target polynucleotide, said target polynucleotide having a sequence of claim 1.

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- 21. An array of claim 20, wherein said first oligonucleotide or polynucleotide sequence is completely complementary to at least 30 contiguous nucleotides of said target polynucleotide.
- 22. An array of claim 20, wherein said first oligonucleotide or polynucleotide sequence is completely complementary to at least 60 contiguous nucleotides of said target polynucleotide
  - 23. An array of claim 20, which is a microarray.
- 24. An array of claim 20, further comprising said target polynucleotide hybridized to said first oligonucleotide or polynucleotide.
  - 25. An array of claim 20, wherein a linker joins at least one of said nucleotide molecules to said solid substrate.
  - 26. An array of claim 20, wherein each distinct physical location on the substrate contains multiple nucleotide molecules having the same sequence, and each distinct physical location on the substrate contains nucleotide molecules having a sequence which differs from the sequence of nucleotide molecules at another physical location on the substrate.
  - 27. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:
    - a) an amino acid sequence selected from the group consisting of SEQ ID NO:46-90.
  - b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:46-90,
  - c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:46-90, and
  - d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:46-90.

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      CHALUP, Michael S.
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      FLORES, Vincent
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      HILLMAN, Jennifer L.
      JONES, Anissa L.
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      ROSEBERRY, Ann M.
      ROSEN, Bruce H. RUSSO, Frank D.
      STOCKDREHER, Theresa K.
      DAFFO, Abel
      WRIGHT, Rachel J.
      YAP, Pierre E.
      YU, Jimmy Y.
      BRADLEY, Diana L.
      BRATCHER, Shawn R.
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Glu Gln Thr Val Ala Thr Met Thr Ser Glu Glu Lys Lys Glu Arg
                110
                                     115
Pro Ile Ser Met Ile Asn Glu Ala Ser Asn Tyr Asn Val Thr Ser
                125
                                     130
Asp Tyr Ala Val His Pro Met Ser Pro Val Gly Arg Thr Ser Arg
                140
                                     145
Ala Ser Lys Lys Val His Asn Phe Gly Lys Arg Ser Asn Ser Ile
                155
                                     160
                                                          165
Lys Arg Asn Pro Asn Ala Pro Val Val Arg Arg Gly Trp Leu Tyr
                170
                                     175
                                                          180
Lys Gln Asp Ser Thr Gly Met Lys Leu Trp Lys Lys Arg Trp Phe
                185
                                     190
Val Leu Ser Asp Leu Cys Leu Phe Tyr Tyr Arg Asp Glu Lys Glu
                200
                                     205
Glu Gly Ile Leu Gly Ser Ile Leu Leu Pro Ser Phe Gln Ile Ser
                215
                                     220
Phe Ala Tyr Pro Leu Lys Ile Thr Leu Ile Ala Asn Met Leu Leu
                230
                                     235
Arg Gln Pro Ile Gln Thr Cys Gly Pro Ile Ile Ser Ala Leu Ile
                245
                                     250
Gln Glu Arg Lys Trp Ser Cys Gly
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Ser Leu Pro Ser Thr Ser Phe Arg Val Ser Ser Leu Phe Ser Gly
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His Leu Glu Val Leu Lys Leu Leu Val Ala Arg Gly Ala Asp Leu
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                                      25
Gly Cys Lys Ala Arg Lys Gly Tyr Gly Leu Leu His Thr Ala Ala
                                      40
                                                           45
Ala Ser Gly Gln Ile Glu Val Val Lys Tyr Leu Leu Arg Met Gly
                 50
                                      55
                                                           60
Ala Glu Ile Asp Glu Pro Asn Ala Phe Gly Asn Thr Ala Leu His
                 65
Ile Ala Cys Tyr Leu Gly Gln Asp Ala Val Ala Ile Glu Leu Val
                 80
                                      85
Asn Ala Gly Ala Asn Val Asn Gln Pro Asn Asp Lys Gly Phe Thr
                                     100
                                                          105
Pro Leu His Val Ala Ala Val Ser Thr Asn Gly Ala Leu Cys Leu
                110
                                     115
Glu Leu Leu Val Asn Asn Gly Ala Asp Val Asn Tyr Gln Ser Lys
                125
                                     130
                                                          135
Glu Gly Lys Ser Pro Leu His Met Ala Ala Ile His Gly Arg Phe
                140
                                     145
Thr Arg Ser Gln Ile Leu Ile Gln Asn Gly Ser Glu Ile Asp Cys
                 155
                                     160
                                                          165
Ala Asp Lys Phe Gly Asn Thr Pro Leu His Val Ala Ala Arg Tyr
                170
                                     175
                                                          180
Gly His Glu Leu Leu Ile Ser Thr Leu Met Thr Asn Gly Ala Asp
                185
                                     190
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Thr Gly Arg Arg Gly Ile His Asp Met Phe Pro Leu His Leu Ala
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                                     205
Val Leu Phe Gly Phe Ser Asp
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Gln Arg Gly Ala Lys Thr Arg Leu Arg Pro Phe Ser Pro Arg His
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Cys Tyr Lys Ala Ala Thr Ile Lys Asp Val Phe Gly Arg Asn Ala
Leu His Pro Cys Phe Leu Leu Val Glu Lys Lys Gly Val Leu Asp
                 35
                                      40
Trp Leu Ile Gln Lys Gly Val Asp Leu Leu Val Lys Asp Lys Glu
                 50
                                      55
                                                           60
Ser Gly Trp Thr Ala Leu His Arg Ser Ile Phe Tyr Gly His Ile
                 65
                                      70
Asp Cys Val Trp Ser Leu Leu Lys His Gly, Val Ser Leu Tyr Ile
                 80
                                      85
                                                           90
Gln Asp Lys Glu Gly Leu Ser Ala Leu Asp Leu Val Met Lys Asp
                 95
                                     100
Arg Pro Thr His Val Val Phe Lys Asn Thr Asp Pro Thr Asp Val
                110
                                     115
                                                          120
Tyr Thr Trp Gly Asp Asn Thr Asn Phe Thr Leu Gly His Gly Ser
                125
                                     130
                                                          135
Gln Asn Ser Lys His His Pro Glu Leu Val Asp Leu Phe Ser Arg
                140
                                     145
                                                          150
Ser Gly Ile Tyr Ile Lys Gln Val Val Leu Cys Lys Phe His Ser
                155
                                     160
                                                          165
Val Phe Leu Ser Gln Lys Gly Gln Val Tyr Thr Cys Gly His Gly
                170
                                     175
                                                          180
Pro Gly Gly Arg Leu Gly His Gly Asp Glu Gln Thr Cys Leu Val
                185
                                     190
                                                          195
Pro Arg Leu Val Glu Gly Leu Asn Gly His Asn Cys Ser Gln Val
                200
                                     205
                                                          210
Ala Ala Ala Lys Asp His Thr Val Val Leu Thr Glu Asp Gly Cys
                215
Val Tyr Thr Phe Gly Leu Asn Ile Phe His Gln Leu Gly Ile Ile
                230
                                     235
                                                          240
Pro Pro Pro Ser Ser Cys Asn Val Pro Arg Gln Ile Gln Ala Lys
                245
                                     250
                                                          255
Tyr Leu Lys Gly Arg Thr Ile Ile Gly Val Ala Ala Gly Arg Phe
                260
                                     265
                                                          270
His Thr Val Leu Trp Thr Arg Glu Ala Val Tyr Thr Met Gly Leu
                275
                                     280
                                                          285
Asn Gly Gly Gln Leu Gly Cys Leu Leu Asp Pro Asn Gly Glu Lys
                290
                                     295
Cys Val Thr Ala Pro Arg Gln Val Ser Ala Leu His His Lys Asp
                305
                                     310
                                                          315
Ile Ala Leu Ser Leu Val Ala Ala Ser Asp Gly Ala Thr Val Cys
                320
                                     325
                                                          330
Val Thr Thr Arg Gly Asp Ile Tyr Leu Leu Ala Asp Tyr Gln Cys
                335
                                     340
Lys Lys Met Ala Ser Lys Gln Leu Asn Leu Lys Lys Val Leu Val
                350
                                     355
                                                          360
Ser Gly Gly His Met Glu Tyr Lys Val Asp Pro Glu His Leu Lys
                365
                                     370
Glu Asn Gly Gly Gln Lys Ile Cys Ile Leu Ala Met Asp Gly Ala
                380
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                                     385
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Gly Arg Val Phe Cys Trp Arg Ser Val Asn Ser Ser Leu Lys Gln
                  395
                                       400
 Cys Arg Trp Ala Tyr Pro Arg Gln Val Phe Ile Ser Asp Ile Ala
                  410
                                       415
                                                           420
 Leu Asn Arg Asn Glu Ile Leu Phe Val Thr Gln Asp Gly Glu Gly
                  425
                                       430
                                                           435
 Phe Arg Gly Arg Trp Phe Glu Glu Lys Arg Lys Ser Ser Glu Lys
                  440
                                      445
 Lys Glu Ile Leu Ser Asn Leu His Asn Ser Ser Ser Asp Val Ser
                  455
                                      460
                                                           465
 Tyr Val Ser Asp Ile Asn Ser Val Tyr Glu Arg Ile Arg Leu Glu
                  470
                                       475
 Lys Leu Thr Phe Ala His Arg Ala Val Ser Val Ser Thr Asp Pro
                  485
                                      490
                                                           495
 Ser Gly Cys Asn Phe Ala Ile Leu Gln Ser Asp Pro Lys Thr Ser
                  500
                                      505
                                                           510
 Leu Tyr Glu Ile Pro Ala Val Ser Ser Ser Ser Phe Phe Glu Glu
                  515
                                      520
                                                           525
 Phe Gly Lys Leu Leu Arg Glu Ala Asp Glu Met Asp Ser Ile His
                  530
                                       535
                                                           540
 Asp Val Thr Phe Gln Val Gly Asn Arg Leu Phe Pro Ala His Lys
                  545
                                       550
                                                           555
 Tyr Ile Leu Ala Val His Ser Asp Phe Phe Gln Lys Leu Phe Leu
                  560
                                      565
 Ser Asp Gly Asn Thr Ser Glu Phe Thr Asp Ile Tyr Gln Lys Asp
                  575
                                      580
                                                           585
 Glu Asp Ser Ala Gly Cys His Leu Phe Val Val Glu Lys Val His
                  590
                                      595
 Pro Asp Met Phe Glu Tyr Leu Leu Gln Phe Ile Tyr Thr Asp Thr
                  605
                                      610
                                                           615
. Cys Asp Phe Leu Thr His Gly Phe Lys Pro Arg Ile His Leu Asn
                  620
                                      625
 Lys Asn Pro Glu Glu Tyr Gln Gly Thr Leu Asn Ser His Leu Asn
                                      640
                                                           645
 Lys Val Asn Phe His Glu Asp Asn Gln Lys Ser Ala Phe Glu
                  650
                                       655
 Val Tyr Lys Ser Asn Gln Ala Gln Thr Val Ser Glu Arg Gln Lys
                  665
                                      670
 Ser Lys Pro Lys Ser Cys Lys Xaa Gly Lys Asn Ile Arg Glu Asp
                  680
                                      685
                                                           690
 Asp Pro Val Arg Met Leu Gln Thr Val Ala Lys Lys Phe Asp Phe
                  695
                                      700
 Ser Asn Leu Ser Ser Arg Leu Asp Gly Val Arg
                  710
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 Glu Pro Leu Ser Pro Pro Gly Arg Ile Pro Gly Ala Ala Gly Glu
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 Cys Glu Gly Pro Gln Gly Xaa Phe Ala Ser Arg Gln Pro Tyr Ser
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 Arg Phe Leu Leu Arg Tyr Trp His Leu Thr Pro Ile Thr Pro Trp
                   35
                                       40
                                                            45
 Ala Ile Val Pro Val Trp Ser Pro Arg Gly Arg Ser Arg Gly Ser
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50
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                                                           60
Pro Asn Ser Thr Ser Gln Thr Ser Ile Gln Ala Gly Thr Ser Thr
Leu Leu Ala Ser Arg His Gln Asn Ile Trp Glu Asp Met Cys
                                                         Val
                  80
                                      85
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Ser Thr Cys Met Trp Gly His Thr Gly Gly Asn Met Gly Met Arg
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Ala Val
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Leu Gln Gly Gln Ser Gly Ala Asp Met Asp Lys Arg Val Lys Lys
Leu Pro Leu Met Ala Leu Ser Thr Thr Met Ala Glu Ser Phe Lys
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Glu Leu Asp Pro Asp Ser Ser Met Gly Lys Ala Leu Glu Met Ser
                 35
                                      40
Cys Ala Ile Gln Asn Gln Leu Ala Arg Ile Leu Ala Glu Phe Glu
                  50
                                      55
                                                           60
Met Thr Leu Glu Arg Asp Val Leu Gln Pro Leu Ser Arg Leu Ser
                 65
                                      70
Glu Glu Glu Leu Pro Ala Ile Leu Lys His Lys Lys Ser Leu Gln
                 80
                                      85
Lys Leu Val Ser Asp Trp Asn Thr Leu Lys Asn Arg Leu Ser Gln
                 95
                                     100
Ala Thr Lys Asn Ser Gly Ser Ser Gln Gly Leu Gly Gly Ser Pro
                 110
                                     115
                                                          120
Gly Ser His Ser His Thr Thr Met Ala Asn Lys Val Glu Thr Leu
                125
                                     130
                                                          135
Phe Tyr Cys Ser Arg Xaa Ser Pro Arg Lys Val Glu Gln Cys Arg
                 140
                                     145
Asp Glu Tyr Leu Ala Asp Leu Tyr His Phe Val Thr Lys Glu Asp
                 155
                                     160
                                                          165
Ser Tyr Ala Asn Tyr Phe Ile Arg Leu Leu Glu Ile Gln Ala Asp
                 170
                                     175
                                                          180
Tyr His Arg Arg Ser Leu Ser Ser Leu Asp Thr Ala Leu Ala Glu
                185
                                     190
                                                          195
Leu Arg Glu Asn His Gly Gln Ala Asp His Ser Pro Ser Met Thr
                200
                                     205
Ala Thr His Phe Pro Arg Val Tyr Gly Val Ser Leu Ala Thr His
                215
                                     220
Leu Gln Glu Leu Gly Arg Glu Ile Ala Leu Pro Ile Glu Ala Cys
                 230
                                     235
                                                          240
Val Met Met Leu Leu Ser Glu Gly Met Lys Glu Glu Gly Leu Phe
                245
                                     250
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Arg Leu Ala Ala Gly Ala Ser Val Leu Lys Arg Leu Lys Gln Thr
                260
                                     265
                                                          270
Met Ala Ser Asp Pro His Ser Leu Glu Glu Phe Cys Ser Asp Pro
                275
                                     280
                                                          285
His Ala Val Ala Gly Ala Leu Lys Ser Tyr Leu Arg Glu Leu Pro
                 290
                                     295
                                                          300
Glu Pro Leu Met Thr Phe Asp Leu Tyr Asp Asp Trp Met Arg Ala
                305
                                     310
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Ala Ser Leu Lys Glu Pro Gly Ala Arg Leu Gln Ala Leu Gln Glu

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325
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Val Cys Ser Arg Leu Pro Pro Glu Asn Leu Ser Asn Leu Arg Tyr
                335
                                     340
                                                          345
Leu Met Lys Phe Leu Ala Arg Leu Ala Glu Glu Gln Glu Val Asn
                350
                                     355
                                                          360
Lys Met Thr Pro Ser Asn Ile Ala Ile Val Leu Gly Pro Asn Leu
                                     370
                365
Leu Trp Pro Pro Glu Lys Glu Gly Asp Gln Ala Gln Leu Asp Ala
                                     385
                380
                                                          390
Ala Ser Val Ser Ser Ile Gln Val Val Gly Val Val Glu Ala Leu
                395
                                     400
Ile Gln Ser Ala Asp Thr Leu Phe Pro Gly Asp Ile Asn Phe Asn
                410
                                     415
                                                          420
Val Ser Gly Leu Phe Ser Ala Val Thr Leu Gln Asp Thr Val Ser
                425
                                     430
                                                          435
Asp Arg Leu Ala Ser Glu Glu Leu Pro Ser Thr Ala Val Pro Thr
                440
                                     445
                                                          450
Pro Ala Thr Thr Pro Ala Pro Ala Pro Ala Pro Ala Pro Ala Pro
                455
                                     460
                                                          465
Ala Pro Ala Leu Ala Ser Ala Ala Thr Lys Glu Arg Thr Glu Ser
                470
                                     475
Glu Val Pro Pro Arg Pro Ala Ser Pro Lys Val Thr Arg Ser Pro
                485
                                     490
                                                          495
Pro Glu Thr Ala Ala Pro Val Glu Asp Met Ala Arg Arg Thr Lys
                500
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Arg Pro Ala Pro Ala Arg Pro Thr Met Pro Pro Pro Gln Val Ser
                515
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Gly Ser Arg Ser Ser Pro Pro Ala Pro Pro Leu Pro Pro Gly Ser
                530
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Gly Ser Pro Gly Thr Pro Gln Ala Leu Pro Arg Arg Leu Val Gly
                545
                                     550
                                                          555
Ser Ser Leu Arg Ala Pro Thr Val Pro Pro Pro Leu Pro Pro Thr
                560
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                                     565
Pro Pro Gln Pro Ala Arg Arg Gln Ser Arg Arg Ser Pro Ala Ser
                                     580
                575
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Pro Ser Pro Ala Ser Pro Gly Pro Ala Ser Pro Ser Pro Val Ser
                590
                                     595
                                                          600
Leu Ser Asn Pro Ala Gln Val Asp Leu Gly Ala Ala Thr Ala Glu
                605
                                     610
                                                          615
Gly Gly Ala Pro Glu Ala Ile Ser Gly Val Pro Thr Pro Pro Ala
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Ile Pro Pro Gln Pro Arg Pro Arg Ser Leu Ala Ser Glu Thr Asn
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Gln Leu Ser Arg Gly Leu Ala Leu Phe Trp Ser Pro Arg Pro Asn
Pro Pro Glu Glu Met Ser Gly Gly Leu Ala Pro Ser Lys Ser Thr
                 35
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Val Tyr Val Ser Asn Leu Pro Phe Ser Leu Thr Asn Asn Asp Leu
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Tyr Arg Ile Phe Ser Lys Tyr Gly Lys Val Val Lys Val Thr Ile
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Met Lys Asp Lys Asp Thr Arg Lys Ser Lys Gly Val Ala Phe Ile

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80
Leu Phe Leu Asp Lys Asp Ser Ala Gln Asn Cys Thr Arg Ala Ile
                                     100
                 95
Asn Asn Lys Gln Leu Phe Gly Arg Val Ile Lys Ala Ser Ile Ala
                110
                                     115
                                                         120
Ile Asp Asn Gly Arg Ala Ala Glu Phe Ile Arg Arg Asn
                                                         Tyr
                125
                                     130
                                                         135
Phe Asp Lys Ser Lys Cys Tyr Glu Cys Gly Glu Ser Gly His Leu
                                     145
                140
Ser Tyr Ala Cys Pro Lys Asn Met Leu Gly Glu Arg Glu Pro Pro
                155
                                     160
                                                         165
Lys Lys Lys Glu Lys Lys Glu Lys Lys Glu Ser Ser
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                                     175
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Glu Thr Ser Leu Arg Ser Gly Gln Ile Pro Thr Leu Asp Ser Ser
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Glu His Asn Leu Ser Pro Glu Pro Leu Glu Leu Asp Arg Met Pro
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His Ser Pro Leu Ile Ser Ile Pro His Val Trp Cys His Pro Glu
                                      40
                                                          45
Glu Glu Glu Arg Met His Asp Glu Leu Leu Gln Ala Val Ser Lys
                 50
                                      55
                                                           60
Gly Pro Val Met Phe Arg Asp Val Ser Ile Asp Phe Ser Gln Glu
                 65
                                      70
Glu Trp Glu Cys Leu Asp Ala Asp Gln Met Asn Leu Tyr Lys Glu
                 80
                                      85
Val Met Leu Glu Asn Phe Ser Asn Leu Val Ser Val Gly Leu Ser
                 95
                                     100
Asn Ser Lys Pro Ala Val Ile Ser Leu Leu Glu Gln Gly Lys Glu
                110
                                     115
                                                         120
Pro Trp Met Val Asp Arg Glu Leu Thr Arg Gly Leu Cys Ser Asp
                125
                                     130
                                                         135
Leu Glu Ser Met Cys Glu Thr Lys Ile Leu Ser Leu Lys Lys Arg
                140
                                                         150
His Phe Ser Gln Val Ile Ile Thr Arg Glu Asp Met Ser Thr Phe
                155
                                     160
                                                         165
Ile Gln Pro Thr Phe Leu Ile Pro Pro Gln Lys Thr Met Ser Glu
                170
                                     175
Glu Lys Pro Trp Glu Cys Lys Ile Cys Gly Lys Thr Phe Asn Gln
                185
                                     190
                                                         195
Asn Ser Gln Phe Ile Gln His Gln Arg Ile His Phe Gly Glu Lys
                200
                                     205
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His Tyr Glu Ser Lys Glu Lys
                215
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Ala Gly Cys Gly Trp Asp Pro Val Phe Pro Ala Pro Arg Gly Thr
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Trp Phe Leu Cys Pro Gly Phe Cys His Ser Val Thr Tyr Ala Met
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Pro Cys Cys Ser His Arg Arg Cys Arg Glu Asp Pro Gly Thr Ser
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Glu Ser Gln Glu Met Asp Pro Val Ala Phe Asp Asp Val Ala Val
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                                                           60
Asn Phe Thr Gln Glu Glu Trp Ala Leu Leu Asp Ile Ser Gln Arg
                                      70
                 65
Lys Leu Tyr Lys Glu Val Met Leu Glu Thr Phe Arg Asn Leu Thr
                                      85
                 80
                                                          90
Ser Val Gly Lys Ser Trp Lys Asp Gln Asn Ile Glu Tyr Glu Tyr
                 95
                                     100
                                                          105
Gln Asn Pro Arg Arg Asn Phe Arg Ser Leu Ile Glu Lys Lys Val
                110
                                     115
                                                         120
Asn Glu Ile Lys Asp Asp Ser His Cys Gly Glu Thr Phe Thr Gln
                125
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Val Pro Asp Asp Arg Leu Asn Phe Gln Glu Lys Lys Ala Ser Pro
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His Thr Glu Ala Arg Pro Pro Arg Arg Glu Ser Trp Ile Ser Asp
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Ile Arg Ala Gly Thr Ala Pro Ser Cys Arg Asn His Ile Lys Ser
                 20
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Ser Cys Ser Leu Ile Ala Phe Asn Ser Asp Arg Pro Gly Val Leu
                 35
                                      40
Gly Ile Val Pro Leu Gln Gly Gln Gly Glu Asp Lys Arg Arg Val
                 50
                                      55
Ala His Leu Gly Cys His Ser Asp Leu Val Thr Asp Leu Asp Phe
                                      70
                 65
Ser Pro Phe Asp Asp Phe Leu Leu Ala Thr Gly Ser Ala Asp Arg
                 80
                                                          90
                                      85
Thr Val Lys Leu Trp Arg Leu Pro Gly Pro Gly Gln Ala Leu Pro
                 95
                                     100
Ser Ala Pro Gly Val Val Leu Gly Pro Glu Asp Leu Pro Val Glu
                110
                                     115
                                                          120
Val Leu Gln Phe His Pro Thr Ser Asp Gly Ile Leu Ser Trp Gln
                125
                                     130
                                                          135
Pro Met Gly Thr Trp Cys Arg Ala Pro Ser Gly Ala Glu Met Glu
                140
                                     145
                                                          150
Pro Trp Trp Ala Arg Arg Ala Arg Thr Ser Ser Cys Gly Ser Leu
                155
                                     160
                                                          165
Thr Pro Glu Gln Ser Arg Gly Pro Leu Arg Ala Arg Arg Pro Met
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Arg Thr Ala Gly Ile Ala Gly Trp His Gly Trp Ala Pro
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Ser Gln Pro Ala Ser Gln Thr Gly Leu Arg Pro Thr Asp Gly Arg
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Ser Arg Ser Gly Pro Ala Arg Leu Leu Cys Pro Gly Pro Ala Ala
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Pro Arg Ser Pro Ala Val Ser Ala Ala Ser Arg Pro Glu Ser Gln
                 50
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Ala Pro Thr Pro Arg Pro Ala Val Ala Ala Pro Ser Met Ser Ser
                 65
                                                          75
                                      70
Thr Glu Arg Arg Pro Ala Gly Arg Arg Asp Arg Ser Pro Arg Gln
                 80
                                      85
                                                           90
Gln Val Asp Arg Leu Leu Val Gly Leu Arg Trp Arg Arg Leu Glu
                 95
                                     100
                                                         105
Glu Pro Leu Gly Phe Ile Lys Val Leu Gln Trp Leu Phe Ala Ile
                110
                                     115
Phe Ala Phe Gly Ser Cys Gly Ser Tyr Ser Gly Glu Thr Gly Ala
                125
                                     130
                                                         135
Met Val Arg Cys Asn Asn Glu Ala Lys Asp Val Ser Ser Ile Ile
                140
                                     145
                                                         150
Val Ala Phe Gly Tyr Pro Cys Arg Leu His Arg Ile Gln Tyr Glu
                155
                                     160
Met Pro Leu Cys Asp Glu Glu Ser Ser Lys Thr Met His Leu
                170
                                     175
                                                         180
Met Gly Asp Phe Ser Ala Pro Ala Glu Phe Phe Val Thr Leu Gly
                185
                                     190
                                                          195
Ile Phe Ser Phe Phe Tyr Thr Met Ala Ala Leu Val Ile Tyr Leu
                200
                                     205
                                                         210
Arg Phe His Asn Leu Tyr Thr Glu Asn Lys Arg Phe Pro Leu Val
                215
                                                         225
                                     220
Asp Phe Cys Val Thr Val Ser Phe Thr Phe Phe Trp Leu Val Ala
                230
                                     235
Ala Ala Arp Gly Lys Gly Leu Thr Asp Val Lys Gly Ala Thr
                245
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Arg Pro Ser Ser Leu Thr Ala Ala Met Ser Val Cys His Gly Glu
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Glu Ala Val Cys Ser Ala Gly Ala Thr Pro Ser Met
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Val Val Ser Ser Thr Thr Ala Ser Ala Leu Gln Ser Gln Ser Lys
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Ala Leu Leu Gln Met Lys Ser Gln Glu Glu Val Glu Val Ala Gly
                 20
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Ile Lys Leu Cys Lys Ala Met Ser Leu Gly Ser Leu Thr Phe Thr
                 35
                                      40
Asp Val Ala Ile Asp Phe Ser Gln Asp Glu Trp Glu Trp Leu Asn
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Leu Ala Gln Arg Ser Leu Tyr Lys Lys Val Met Leu Glu Asn Tyr
                                      70
Arg Asn Leu Val Ser Val Gly Leu Cys Ile Ser Lys Pro Asp Val
                 80
                                      85
Ile Ser Leu Leu Glu Gln Glu Lys Asp Pro Trp Val Ile Lys Gly
                 95
                                     100
                                                         105
Gly Met Asn Arg Gly Leu Cys Pro Asp Leu Glu Cys Val Trp Val
                110
                                     115
Thr Lys Ser Leu Ser Leu Asn Gln Asp Ile Tyr Glu Glu Lys Leu
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130
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                125
Pro Pro Ala Ile Ile Met Glu Arg Leu Lys Ser Tyr Asp Leu Glu
                                     145
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                                                          150
Cys Ser Thr Leu Gly Lys Asn Trp Lys Cys Glu Asp Leu Phe Glu
                155
                                     160
Arg Glu Leu Val Asn Gln Lys Thr His Phe Arg Gln Glu Thr Ile
                                     175
                170
                                                          180
Thr His Ile Asp Thr Leu Ile Glu Lys Arg Asp His Ser Asn Lys
                185
                                     190
                                                          195
Ser Gly Thr Val Phe His Leu Asn Thr Leu Ser Tyr Ile Lys Gln
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Ile
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Arg Arg Gln Leu Gly Val Ala Leu Ile Pro Ser His Arg Met Asp
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Tyr Lys Ser Ser Leu Ile Gln Asp Gly Asn Pro Met Glu Asn Leu
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                                                           30
Glu Lys Gln Leu Ile Cys Pro Ile Cys Leu Glu Met Phe Thr Lys
                 35
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                                                           45
Pro Val Val Ile Leu Pro Cys Gln His Asn Leu Cys Arg Lys Cys
                 50
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Ala Asn Asp Ile Phe Gln Ala Ser Asn Pro Tyr Leu Pro Thr Arg
                 65
                                      70
Gly Gly Thr Thr Met Ala Ser Gly Gly Arg Phe Arg Cys Pro Ser
                 80
                                      85
Cys Arg His Glu Val Val Leu Asp Arg His Gly Val Tyr Gly Leu
                 95
                                     100
Gln Arg Asn Leu Leu Val Glu Asn Ile Ile Asp Ile Tyr Lys Gln
                110
                                     115
                                                          120
Glu Cys Ser Ser Arg Pro Leu Gln Lys Gly Ser His Pro Met Cys
                125
                                     130
                                                          135
Lys Glu His Glu Asp Glu Lys Ile Asn Ile Tyr Cys Leu Thr Cys
                140
                                     145
                                                          150
Glu Val Pro Thr Cys Ser Met Cys Lys Val Phe Gly Ile His Lys
                155
                                     160
                                                          165
Ala Cys Glu Val Ala Pro Leu Gln Ser Val Phe Gln Gly Gln Lys
                                     175
                                                          180
Thr Glu Leu Asn Asn Cys Ile Ser Met Leu Val Ala Gly Asn Asp
                185
                                     190
                                                          195
Arg Val Gln Thr Ile Ile Thr Gln Leu Glu Asp Ser Arg Arg Val
                200
                                     205
Thr Lys Glu Asn Ser His Gln Val Lys Glu Glu Leu Ser Gln Lys
                215
                                     220
                                                          225
Phe Asp Thr Leu Tyr Ala Ile Leu Asp Glu Lys Lys Ser Glu Leu
                230
                                     235
                                                          240
Leu Gln Arg Ile Thr Gln Glu Gln Glu Lys Lys Leu Ser Phe Ile
                                     250
                245
                                                          255
Glu Ala Leu Ile Gln Gln Tyr Gln Glu Gln Leu Asp Lys Ser Thr
                260
                                     265
                                                          270
Lys Leu Val Glu Thr Ala Ile Gln Ser Leu Asp Glu Pro Gly Gly
                275
                                     280
                                                          285
Ala Thr Phe Leu Leu Thr Ala Lys Gln Leu Ile Lys Ser Ile Val
                290
                                     295
                                                          300
Glu Ala Ser Lys Gly Cys Gln Leu Gly Lys Thr Glu Gln Gly Phe
                305
                                     310
Glu Asn Met Asp Phe Phe Thr Leu Asp Leu Glu His Il'e Ala Asp
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320
                                    325
Ala Leu Arg Ala Ile Asp Phe Gly Thr Asp Glu Glu Glu Glu Glu
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Lys Glu Glu Gly His Gln
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Glu Met Ala Val Gly Asn Asn Thr Gln Arg Ser Tyr Ser Ile Ile
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Pro Cys Phe Ile Phe Val Glu Leu Val Ile Met Ala Gly Thr Val
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Leu Leu Ala Tyr Tyr Phe Glu Cys Thr Asp Thr Phe Gln Val His
                                     40
                                                         45
Ile Gln Gly Phe Phe Cys Gln Asp Gly Asp Leu Met Lys Pro Tyr
                 50
                                                         60
Pro Gly Thr Glu Glu Glu Ser Phe Ile Thr Pro Leu Val Leu Tyr
                 65
                                     70
Cys Val Leu Ala Ala Thr Pro Thr Ala Ile Ile Phe Ile Gly Glu
                 80
                                     85
Ile Ser Met Tyr Phe Ile Lys Ser Thr Arg Glu Ser Leu Ile Ala
                 95
                                    100
                                                        105
Gln Glu Lys Thr Ile Leu Thr Gly Glu Cys Cys Tyr Leu Asn Pro
                110
                                    115
                                                        120
Leu Leu Arg Arg Ile Ile Arg Phe Thr Gly Val Phe Ala Phe Gly
                125
                                    130
                                                        135
Leu Phe Ala Thr Asp Ile Phe Val Asn Ala Gly Gln Val Val Thr
                140
                                    145
                                                        150
Gly His Leu Thr Pro Tyr Phe Leu Thr Val Cys Lys Pro Asn Tyr
                155
                                    160
                                                        165
Thr Ser Ala Asp Cys Gln Ala His His Gln Phe Ile Asn Asn Gly
                170
                                    175
Asn Ile Cys Thr Gly Asp Leu Glu Val Ile Glu Lys Ala Arg Arg
                185
                                    190
                                                        195
Ser Phe Pro Ser Lys His Ala Ala Leu Ser Ile Tyr Ser Ala Leu
                200
                                    205
                                                        210
Tyr Ala Thr Met Tyr Ile Thr Ser Thr Ile Lys Thr Lys Ser Ser
                215
                                    220
                                                        225
Arg Leu Ala Lys Pro Val Leu Cys Leu Gly Thr Leu Cys Thr Ala
                230
                                    235
                                                        240
Phe Leu Thr Gly Leu Asn Arg Val Ser Glu Tyr Arg Asn His Cys
                245
                                    250
Ser Asp Val Ile Ala Gly Phe Ile Leu Gly Thr Ala Val Ala Leu
                260
                                    265
                                                        270
Phe Leu Gly Met Cys Val Val His Asn Phe Lys Gly Thr Gln Gly
                275
                                    280
                                                        285
Ser Pro Ser Lys Pro Lys Pro Glu Xaa Pro Arg Gly Val Pro Leu
                290
                                    295
                                                        300
Met Ala Phe Pro Arg Ile Glu Ser Pro Leu Glu Thr Leu Ser Ala
                305
                                    310
                                                        315
Gln Asn His Ser Ala Ser Met Thr Glu Val Thr
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Ile Met Glu Glu Lys Gln Gln Ile Ile Leu Ala Asn Gln Asp Gly
                                                          30
                 20
                                      25
Gly Thr Val Ala Gly Ala Ala Pro Thr Phe Phe Val Ile Leu Lys
                 35
                                      40
                                                           45
Gln Pro Gly Asn Gly Lys Thr Asp Gln Gly Ile Leu Val Thr Asn
                 50
                                      55
Gln Asp Ala Cys Ala Leu Ala Ser Ser Val Ser Ser Pro Val Lys
                                      70
                 65
                                                           75
Ser Lys Gly Lys Ile Cys Leu Pro Ala Asp Cys Thr Val Gly Gly
                 80
                                      85
                                                           90
Ile Thr Val Thr Leu Asp Asn Asn Ser Met Trp Asn Glu Phe Tyr
                 95
                                     100
                                                          105
His Arg Ser Thr Glu Met Ile Leu Thr Lys Gln Gly Arg Arg Met
                110
                                     115
                                                         120
Phe Pro Tyr Cys Arg Tyr Trp Ile Thr Gly Leu Asp Ser Asn Leu
                125
                                     130
Lys Tyr Ile Leu Val Met Asp Ile Ser Pro Val Asp Asn His Arg
                                     145
                140
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Tyr Lys Trp Asn Gly Arg
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Ser Ser Pro Thr Ser Trp Arg Ser Ser Met Pro Cys Thr Trp Arg
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Ser Arg Arg Arg Cys Thr Ala Cys Ser Ala Ala Ala Pro
                 20
                                      25
Pro Leu Pro Ala Gln Lys Val Cys Leu Arg Cys Glu Ala Pro Cys
                 35
                                      40
                                                           45
Cys Gln Ser His Val Gln Thr His Leu Gln Gln Pro Ser Thr Ala
                 50
                                      55
Arg Gly His Leu Leu Val Glu Ala Asp Asp Val Arg Ala Trp Ser
                                      70
                 65
                                                           75
Cys Pro Gln His Asn Ala Tyr Arg Leu Tyr His Cys Glu Ala Glu
                 80
                                      85
                                                           90
Gln Val Ala Val Cys Gln Tyr Cys Cys Tyr Tyr Ser Gly Ala His
                 95
                                     100
                                                          105
Gln Gly His Ser Val Cys Asp Val Glu Ile Arg Arg Asn Glu Ile
                110
                                     115
Arg Lys Met Leu Met Lys Gln Gln Asp Arg Leu Glu Glu Arg Glu
                125
                                     130
                                                         135
Gln Asp Ile Glu Asp Gln Leu Tyr Lys Leu Glu Ser Asp Lys Arg
                140
                                     145
                                                          150
Leu Val Glu Glu Lys Val Asn Gln Leu Lys Glu Glu Val Arg Leu
                155
                                     160
                                                          165
Gln Tyr Glu Lys Leu His Gln Leu Leu Asp Glu Asp Leu Arg Gln
                170
                                                         180
                                     175
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Thr Val Glu Val Leu Asp Lys Ala Gln Ala Lys Phe Cys Ser Glu
Asn Ala Ala Gln Ala Leu His Leu Gly Glu Arg Met Gln Glu Ala
                 200
                                      205
Lys Lys Leu Leu Gly Ser Leu Gln Leu Leu Phe Asp Lys Thr Glu
                 215
                                     220
Asp Val Ser Phe Met Lys Asn Thr Lys Ser Val Lys Ile Leu Met
                 230
                                     235
                                                          240
Asp Ser Arg Cys Pro Val His Trp Pro Gln Asp Pro Asp Leu His
                 245
                                     250
Glu Gln Gln Pro Phe Pro His
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Lys Thr Asn Leu Tyr Cys Ser Pro Tyr Phe Ile Asp Cys Asn Arg
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Ser Ile Glu Val Thr Phe Ile Leu Ser Trp Ile Val Cys Ser Tyr
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                                      25
Ala Val Cys Lys Glu Arg Asn Gly Met Gly Gly Cys Glu Lys Glu
                  35
                                      40
                                                           45
Glu Leu Val Val Asp Phe Gly Gly Ala Gly Trp Arg Ser Leu Cys
                                      55
                  50
                                                           60
Leu Cys Ser Arg Leu Gly Cys Ala Ala Pro Arg Pro Arg Cys Pro
                                                           75
Asp Phe Arg Arg Pro Asp Ala Ser Leu Thr Ser Ala Ser Ala Arg
                  80
                                      85
                                                           90
'Gly Cys Trp Arg Pro Ser Trp Leu Arg Ser Ala Pro Pro Arg Ser
                  95
                                     100
Pro Pro Thr Thr Cys Ala His Pro Ala Trp Arg Cys Pro Ser Pro
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                                     115
Arg Cys Arg Arg Thr Pro Ala Pro Phe Arg Cys Cys
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Pro Pro Arg Arg Pro Cys Trp Phe Leu Cys Gly Leu Leu Ser
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Arg Met Val Lys Leu Phe Ile Gly Asn Leu Pro Arg Glu Ala Thr
                  20
                                      25
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Glu Gln Glu Ile Arg Ser Leu Phe Glu Gln Tyr Gly Lys Val Leu
                  35
                                      40
                                                           45
Glu Cys Asp Ile Ile Lys Asn Tyr Gly Phe Val His Ile Glu Asp
                  50
                                      55
                                                           60
Lys Thr Ala Ala Glu Asp Ala Ile Arg Asn Leu His His His Lys
                  65
                                      70
                                                           75
Pro His Gly Val Asn Ile Asn Ala Glu Ala Ser Lys Asn Lys Ser
                                                           90
                                      85
Lys Ala Pro Thr Lys Leu His Val Gly Asn Ile Ser Pro Thr Cys
                  95
                                     100
Thr Asn Gln Glu Leu Arg Ala Lys Phe Glu Glu His Gly Pro Ala
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110
                                     115
Ile Glu Cys Asp Ile Ala Lys Asp Tyr Ala Phe Ala His Met Glu
                125
                                     130
                                                          135
Arg Ala Glu Asp Ala Ala Glu Ala Ile Arg Gly Leu Asp Asn Thr
                140
                                     145
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Glu Phe Gln Gly Glu Leu Leu Trp Ala Trp Val Val Ala Pro Ser
                                     160
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Gly Val
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His Met Thr Gly Pro Met Cys Leu Ile Glu Asn Thr Asn Gly Glu
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Leu Val Ala Asn Pro Glu Ala Leu Lys Ile Leu Ser Ala Ile Thr
                                                           45
                                      40
Gln Pro Val Val Val Ala Ile Val Gly Leu Tyr Arg Thr Gly
                 50
                                                           60
Lys Ser Tyr Leu Met Asn Lys Leu Ala Gly Lys Asn Lys Gly Phe
                 65
                                      70
Ser Leu Gly Ser Thr Val Lys Ser His Thr Lys Gly Ile Trp Met
                 80
                                      85
                                                          90
Trp Cys Val Pro His Pro Lys Lys Pro Glu His Thr Leu Val Leu
                 95
                                     100
                                                          105
Leu Asp Thr Glu Gly Leu Gly Asp Val Lys Lys Gly Asp Asn Gln
                110
                                     115
                                                          120
Asn Asp Ser Trp Ile Phe Thr Leu Ala Val Leu Leu Ser Ser Thr
                125
                                                          135
                                     130
Leu Val Tyr Asn Ser Met Gly Thr Ile Asn Gln Gln Ala Met Asp
                140
                                     145
                                                          150
Gln Leu Tyr Tyr Val Thr Glu Leu Thr His Arg Ile Arg Ser Lys
                155
                                                          165
                                     160
Ser Ser Pro Asp Glu Asn Glu Asp Ser Ala Asp Phe Val
                170
                                     175
                                                          180
Ser Phe Phe Pro Asp Phe Val Trp Thr Leu Arg Asp Phe Ser Leu
                185
                                     190
                                                          195
Asp Leu Glu Ala Asp Gly Gln Pro Leu Thr Pro Asp Glu Tyr Leu
                200
                                     205
                                                          210
Glu Tyr Ser Leu Lys Leu Thr Gln Gly Thr Ser Gln Lys Asp Lys
                215
                                     220
                                                          225
Asn Phe Asn Leu Pro Gln Leu Cys Ile Trp Lys Phe Phe Pro Lys
                230
                                     235
                                                          240
Lys Lys Cys Phe Val Phe Asp Leu Pro Ile His Arg Arg Lys Leu
                245
                                     250
                                                          255
Ala Gln Leu Glu Lys Leu Gln Asp Glu Glu Leu Asp Pro Glu Phe
                260
                                     265
                                                          270
Val Gln Gln Val Ala Asp Phe Cys Ser Tyr Ile Phe Ser Asn Ser
                                     280
                275
                                                          285
Lys Thr Lys Thr Leu Ser Gly Gly Ile Lys Val Asn Gly Pro Arg
                290
                                     295
                                                          300
Leu Glu Ser Leu Val Leu Thr Tyr Ile Asn Ala Ile Ser Arg Gly
                305
                                     310
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Asp Leu Pro Cys Met Glu Asn Ala Val Leu Ala Leu Ala Gln Ile

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320
Glu Asn Ser Ala Ala Val Gln Lys Ala Ile Ala His Tyr Asp Gln
                                     340
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                                                          345
Gln Met Gly Gln Lys Val Gln Leu Pro Ala Glu Thr Leu Gln Glu
                350
                                     355
Leu Leu Asp Leu His Arg Val Ser Glu Arg Glu Ala Thr Glu Val
                                     370
                365
                                                          375
Tyr Met Lys Asn Ser Phe Lys Asp Val Asp His Leu Phe Gln Lys
                380
                                     385
                                                          390
Lys Leu Ala Ala Gln Leu Asp Lys Lys Arg Asp Asp Phe Cys Lys
                395
                                     400
                                                          405
Gln Asn Gln Glu Ala Ser Ser Asp Arg Cys Ser Ala Leu Leu Gln
                410
                                     415
                                                          420
Val Ile Phe Ser Pro Leu Glu Glu Val Lys Ala Gly Ile Tyr
                425
                                     430
                                                          435
Ser Lys Pro Gly Gly Tyr Cys Leu Phe Ile Gln Lys Leu Gln Asp
                440
                                                          450
Leu Glu Lys Lys Tyr Tyr Glu Glu Pro Arg Lys Gly Ile Gln Ala
                455
                                     460
Glu Glu Ile Leu Gln Thr Tyr Leu Lys Ser Lys Glu Ser Val Thr
                470
                                     475
Asp Ala Ile Leu Gln Thr Asp Gln Ile Leu Thr Glu Lys Glu Lys
                485
                                     490
                                                          495
Glu Ile Glu Val Glu Cys Val Lys Ala Glu Ser Ala Gln Ala Ser
                500
                                     505
                                                          510
Ala Lys Met Val Glu Glu Met Gln Ile Lys Tyr Gln Gln Met Met
                515
                                     520
                                                          525
Glu Glu Lys Glu Lys Ser Tyr Gln Glu His Val Lys Gln Leu Thr
                530
                                     535
Glu Lys Met Glu Arg Glu Arg Ala Gln Leu Leu Glu Glu Gln Glu
                545
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Lys Thr Leu Thr Ser Lys Leu Gln Val Ser Lys Cys Lys Xaa Xaa
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Thr Pro Ala Thr Ile Pro Ser Leu Gly Pro Trp Gly Val Leu His
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Ser Asn Pro Met Asp Tyr Ala Trp Gly Ala Asn Gly Leu Asp Ala
                 35
                                      40
Ile Ile Thr Gln Leu Leu Asn Gln Phe Glu Asn Thr Gly Pro Pro
                 50
                                      55
Pro Ala Asp Lys Glu Lys Ile Gln Ala Leu Pro Thr Val Pro Val
                                      70
                 65
Thr Glu Glu His Val Gly Ser Gly Leu Glu Cys Pro Val Cys Lys
                 80
                                                           90
                                      85
Asp Asp Tyr Ala Leu Gly Glu Arg Val Arg Gln Leu Pro Cys Asn
                 95
                                     100
His Leu Phe His Thr Thr Tyr Glu Gln Ala Trp Leu Glu Gln His
                110
                                     115
Asp Ser Cys Pro Val Cys Arg Lys Ser Leu Thr Gly Gln Asn Thr
                125
                                     130
                                                          135
Ala Thr Asn Pro Pro Gly Leu Thr Gly Val Ser Phe Ser Ser Ser
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Ser Ser Ser Ser Ser Ser Ser Pro Ser Asn Glu Asn Ala Thr

PCT/US01/05896

WO 01/62922 155 160 165 Ser Asn Ser <210> 65 <211> 246 <212> PRT <213> Homo sapiens <220> <221> misc\_feature <223> Incyte ID No: LG:1076157.1.orf3:2000MAY19 <220> <221> unsure <222> 240 <223> unknown or other <400> 65 Pro Lys Gln Gly Ile Asn Val Trp Ser Pro Arg His Pro Glu Asn 10 Phe Leu Gly Ile Glu Ser Arg Pro Pro Met Leu Ser Leu Ser Pro 20 25 Ile Leu Leu Tyr Thr Cys Glu Met Phe Gln Asp Pro Val Ala Phe 35 40 Lys Asp Val Ala Val Asn Phe Thr Glu Glu Trp Ala Leu Leu 50 55 60 Asp Ile Ser Gln Arg Lys Leu Tyr Arg Glu Val Met Leu Glu Thr Phe Arg Asn Leu Thr Ser Ile Gly Lys Lys Trp Lys Asp Gln Asn 80 85 Ile Glu Tyr Glu Tyr Gln Asn Pro Arg Arg Asn Phe Arg Ser Leu 95 100 Ile Glu Gly Asn Val Asn Glu Ile Lys Glu Asp Ser His Cys Gly 110 115 120 Glu Thr Phe Thr Gln Val Pro Asp Asp Arg Leu Asn Phe Gln Glu 125 130 135 Lys Lys Ala Ser Pro Glu Ala Lys Ser Cys Asp Asn Phe Val Cys 140 145 150 Gly Glu Val Gly Ile Gly Asn Ser Ser Phe Asn Met Asn Ile Arg 155 160 165 Gly Asp Ile Gly His Lys Ala Tyr Glu Tyr Gln Asp Tyr Ala Pro 175 170 180 Lys Pro Tyr Lys Cys Gln Gln Pro Lys Lys Ala Phe Arg Tyr His 185 190 195 Pro Ser Phe Arg Thr Gln Glu Arg Asn His Thr Gly Glu Lys Pro 200 205 210 Tyr Ala Cys Lys Glu Cys Gly Lys Thr Phe Ile Ser His Ser Gly 215 220 225 Ile Arg Arg Met Val Met His Ser Gly Asp Gly Pro Leu Xaa 230 235 240 Val Ser Phe Val Gly Lys 245 <210> 66 <211> 120 <212> PRT <213> Homo sapiens

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Xaa Phe Pro Val Leu Glu Pro His Gln Val Gly Leu Ile Arg Ser
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Tyr Asn Ser Lys Thr Met Thr Cys Phe Gln Glu Leu Val Thr Phe
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Arg Asp Val Ala Ile Asp Phe Ser Arg Gln Glu Trp Glu Tyr Leu
                 35
                                      40
Asp Pro Asn Gln Arg Asp Leu Tyr Arg Asp Val Met Leu Glu Asn
                 50
                                      55
Tyr Arg Asn Leu Val Ser Leu Gly Gly His Ser Ile Ser Lys Pro
                 65
                                      70
Val Val Val Asp Leu Leu Glu Arg Gly Lys Glu Pro Trp Met Ile
                 80
                                      85
Leu Arg Glu Glu Thr Gln Phe Thr Asp Leu Asp Leu Gln Cys Glu
                 95
                                     100
Ile Ile Ser Tyr Ile Glu Val Pro Thr Tyr Glu Thr Asp Ile Ser
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Lys Lys Ser Gln Lys Glu Ser Thr Gln Gln Thr Arg Ile His Phe
Gln Arg Asp Ile Leu Cys Lys Glu Ala Thr Trp Lys Arg Lys Glu
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                                      25
Lys Lys Ser Gly Met Ala Leu Thr Gln Gly Pro Leu Lys Phe Met
                 35
                                      40
Asp Val Ala Ile Glu Phe Ser Gln Glu Glu Trp Lys Cys Leu Asp
                 50
                                                          60
Pro Ala Gln Arg Thr Leu Tyr Arg Asp Val Met Leu Glu Asn Tyr
                 65
                                      70
Arg Asn Leu Val Ser Leu Gly Ile Cys Leu Pro Asp Leu Ser Val
                 80
                                      85
Thr Ser Met Leu Glu Gln Lys Arg Asp Pro Trp Thr Leu Gln Ser
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Glu Glu Lys Ile Ala Asn Asp Pro Asp Gly Arg Glu Cys Ile Gln
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                                     115
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Lys Val
<210> 68
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Ala Gln Gly Ser Ser Trp Lys Leu Pro Phe Glu Arg Leu Ala Phe
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Val Leu Ser Ser Asn Ser Leu Lys His Cys Thr Glu Leu Glu Leu
                 20
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Phe Lys Ala Thr Cys Arg Trp Leu Arg Leu Glu Glu Pro Arg Met
                 35
                                      40
Asp Phe Ala Ala Lys Leu Met Lys Asn Ile Arg Phe Pro Leu Met
                                      55
Thr Pro Gln Glu Leu Ile Asn Tyr Val Gln Thr Val Asp Phe Met
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70
Arg Thr Asp Asn Thr Cys Val Asn Leu Leu Glu Ala Ser Asn
                 80
Tyr Gln Met Met Pro Tyr Met Gln Pro Val Met Gln Ser Asp Arg
                 95
                                     100
                                                          105
Thr Ala Ile Arg Ser Asp Thr Thr His Leu Val Thr Leu Gly Gly
                                     115
                110
                                                          120
Val Leu Arg Gln Gln Leu Val Val Ser Lys Glu Leu Arg Met Tyr
                                     130
                125
                                                          135
Asp Glu Lys Ala His Glu Trp Lys Ser Leu Ala Pro Met Asp Ala
                 140
                                     145
                                                          150
Pro Arg Tyr Gln His Gly Ile Ala Val Ile Gly Asn Phe Leu Tyr
                 155
                                     160
                                                          165
Val Val Gly Gly Gln Ser Asn Tyr Asp Thr Lys Gly Lys Thr Ala
                170
                                     175
                                                          180
Val Asp Thr Val Phe Arg Phe Asp Pro Arg Tyr Asn Lys Trp Met
                                     190
                                                          195
Gln Val Ala Ser Leu Asn Glu Lys Arg Thr Phe Phe His Leu Ser
                200
                                     205
                                                          210
Ala Leu Lys Gly Tyr Leu Tyr Ala Val Gly Gly Arg Asn Ala Ala
                215
                                     220
                                                          225
Gly Glu Leu Pro Thr Val Glu Cys Tyr Asn Pro Arg Thr Asn Glu
                230
                                     235
                                                          240
Trp Thr Tyr Val Ala Lys Met Ser Glu Pro His Tyr Gly His Ala
                245
                                     250
                                                          255
Gly Thr Val Tyr Gly Gly Val Met Tyr Ile Ser Gly Gly Ile Thr
                260
                                     265
                                                          270
His Asp Thr Phe Gln Lys Glu Leu Met Cys Phe Asp Pro Asp Thr
                 275
                                     280
                                                          285
Asp Lys Trp Ile Gln Lys Ala Pro Met Thr Thr Val Arg Gly Leu
                 290
                                     295
                                                          300
His Cys Met Cys Thr Val Gly Glu Arg Leu Tyr Val Ile Gly Gly
                305
                                     310
                                                          315
Asn His Phe Arg Gly Thr Ser Asp Tyr Asp Asp Val Leu Ser Cys
                320
                                     325
                                                          330
Glu Tyr Tyr Ser Pro Ile Leu Asp Gln Trp Thr Pro Ile Ala Ala
                335
                                     340
                                                          345
Met Leu Arg Gly Gln Ser Asp Val Gly Val Ala Val Phe Glu Asn
                 350
                                     355
                                                          360
Lys Ile Tyr Val Val Gly Gly Tyr Ser Trp Asn Asn Arg Cys Met
                365
                                     370
Val Glu Ile Val Gln Lys Tyr Asp Pro Asp Lys Asp Glu Trp His
                380
                                     385
                                                          390
Lys Val Phe Asp Leu Pro Glu Ser Leu Gly Gly Ile Arg Ala Cys
                395
                                     400
                                                          405
Thr Leu Thr Val Phe Pro Pro Glu Glu Thr Thr Pro Ser Pro Ser
                410
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Arg Glu Ser Pro Leu Ser Ala Pro
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Arg Asp Pro Gly Trp Gln Ile Arg Asp Arg Ala Gly Leu Ala Trp
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Asn Met Leu Ala Asn Ser Ala Ser Val Arg Ile Leu Ile Lys Gly
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                                      25
                                                           30
Gly Lys Val Val Asn Asp Asp Cys Thr His Glu Ala Asp Val
                                                          Tyr
                 35
                                      40
Ile Glu Asn Gly Ile Ile Gln Gln Val Gly Arg Glu Leu Met Ile
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Pro Gly Gly Ala Lys Val Ile Asp Ala Thr Gly Lys Leu Val Ile
                 65
                                      70
Pro Gly Gly Ile Asp Thr Ser Thr His Phe His Gln Thr Phe Met
                 80
                                      85
Asn Ala Thr Cys Val Asp Asp Phe Tyr His Gly Thr Lys Ala Ala
                 95
                                     100
Leu Val Gly Gly Thr Thr Met Ile Ile Gly His Val Leu Pro Asp
                110
                                     115
                                                          120
Lys Glu Thr Ser Leu Val Asp Ala Tyr Glu Lys Cys Arg Gly Leu
                125
                                     130
Ala Asp Pro Lys Val Cys Cys Asp Tyr Ala Leu His Val Gly Ile
                140
                                     145
                                                          150
Thr Trp Trp Ala Pro Lys Val Lys Ala Glu Met Glu Thr Leu Val
                155
                                     160
Arg Glu Lys Gly Val Asn Ser Phe Gln Met Phe Met Thr Tyr Lys
                170
                                     175
                                                          180
Asp Leu Tyr Met Leu Arg Asp Ser Glu Leu Tyr Gln Val Leu His
                185
                                     190
Ala Cys Lys Asp Ile Gly Ala Ile Ala Arg Val His Ala Glu Asn
                200
                                     205
Gly Glu Leu Val Ala Glu Gly Ala Lys Glu Ala Leu Asp Leu Gly
                215
                                     220
                                                          225
Ile Thr Gly Pro Glu Gly Ile Glu Ile Ser Arg Pro Glu Glu Leu
                230
                                     235
Glu Ala Glu Ala Thr His Arg Val Ile Thr Arg Asp Gly Gly Asn
                245
                                     250
                                                          255
His Asp Ala Ala Ser Trp Cys Ser Ala His His Leu Tyr Pro Cys
                260
                                     265
                                                          270
Gln Pro Ser Leu Gly His Gly Pro Trp Ala Asp Val Lys Glu Pro
                275
                                     280
Ser Ser Ser Gly Gly Gly Gln Leu Gly Arg Ala Ser Leu Leu Gly
                290
                                     295
Leu Gly Lys Leu Tyr Leu Leu
                305
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Leu Pro Ser Arg Thr Phe Leu Gln Ala Leu Asn Leu Gly Ile Glu
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Val Ile Asn Thr Thr Asp Tyr Leu His Phe Ser Lys Glu Cys Ser
                 35
                                      40
Arg Ala Leu Leu Lys Met Gln Tyr Cys Pro His Cys Gln Gly Leu
                 50
                                      55
Ala Leu Thr Lys Pro Cys Met Gly Tyr Cys Leu Asn Val Met Arg
                                      70
                 65
                                                           75
Gly Cys Leu Ala His Met Ala Glu Leu Asn Pro His Trp His Ala
                 80
                                      85
Tyr Ile Arg Ser Leu Glu Glu Leu Ser Asp Ala Met His Gly Thr
                 95
                                     100
Tyr Asp Ile Gly His Val Leu Leu Asn Phe His Leu Leu Val Asn
                110
                                     115
Asp Ala Val Leu Gln Ala His Leu Asn Gly Gln Lys Leu Leu Glu
                125
                                                          135
                                     130
Gln Val Asn Arg Ile Cys Gly Arg Pro Val Arg Thr Pro Thr Gln
                140
                                     145
Ser Pro Arg Cys Ser Phe Asp Gln Ser Lys Glu Lys His Gly Met
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155
                                     160
Lys Thr Thr Thr Arg Asn Ser Glu Glu Thr Leu Ala Asn Arg Arg
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                                     175
Lys Glu Phe Ile Asn Ser Leu Ser Thr Val Gln Val Ile Leu Trp
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Arg Ser Ser
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Ala Thr Pro Ser Gly Arg Pro Gln Ser Trp Thr Arg Phe Ser Leu
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Trp Arg Gly Pro Arg Arg Thr Arg Pro Ser Pro Pro Ala Pro Ala
                                      25
Pro Ala Gly Met Gly Ser Glu His Asp Gly Arg Ser Gly Pro Val
                 35
                                      40
Leu Thr Pro Ala Asp Thr Leu His Pro Pro Thr Arg Leu Gln Pro
                 50
                                      55
Ser Pro Pro Asp Thr His Pro Gly Gly Ser Ser Leu Pro Ala Pro
                 65
                                      70
                                                           75
Arg Pro Ala Leu Ser Cys Trp Ala Arg Val Phe Ala Ser Leu Val
                 80
                                      85
                                                           90
Arg Pro Ala Gly Phe Pro Gly Gly Thr His Gly Ala Pro Gly Met
                 95
                                     100
                                                          105
Pro Leu Gly Ser Pro Ser Thr Ser Thr Ala Gln Trp Pro Tyr Val
                110
                                     115
                                                          120
Gln Leu Val Pro Gly Pro Arg Val Arg Lys Thr Ala Ser Arg Ser
                125
                                     130
His Cys Gln Glu Arg Ala Glu Glu Trp Ser Gly Pro Arg Arg Pro
                140
                                     145
                                                          150
Trp Gly Glu Gly Asp Pro Gly Pro Val Thr Ala Thr Pro Gly Thr
                155
                                     160
                                                          165
Pro Gly Gly Ala Pro Thr Ser Ala Phe Ser Cys Ala Ala Lys Leu
                170
                                     175
                                                          180
Gln Lys Pro Asp Ala Gly Leu Val Val Ala Asn Gly Thr Met Cys
                185
                                     190
                                                          195
Cys Pro Ala Lys His Thr Trp Arg Ser Gly Pro Lys Ile Pro Ile
                200
                                     205
                                                          210
Leu Asp Phe His Pro Ala Pro Ser Ser Thr Pro Arg Ser Ala Leu
                215
                                     220
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Ser His
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Ser Val His Phe Ser Arg Lys Gly Phe Val Leu Met Ala Pro Pro
 1
                                      10
Gln Pro Lys Ser Gly Leu Phe Val Gly Ile Asn Lys Gly His Val
                 20
                                      25
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Val Thr Lys Arg Glu Leu Pro Pro Arg Pro Cys His Arg Lys Gly
                 35
                                      40
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Lys Ser Thr Lys Arg Val Ser Met Val Arg Gly Leu Ile Arg Glu
                 50
                                      55
Val Ala Gly Phe Ala Pro Tyr Glu Lys Arg Ile Thr Glu Leu Leu
Lys Val Gly Lys Asp Lys Arg Ala Leu Lys Leu Ala Lys Arg Lys
                 80
                                      85
Leu Gly Thr His Lys Arg Ala Lys Lys Lys Arg Glu Glu Met Ala
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                                     100
Gly Val Leu Arg Lys Met Arg Ser Ala Gly Thr His Thr Asp Lys
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Lys Lys
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Cys Ser Gln Ile Glu Leu Ala Ile Glu Leu Asp Ser Thr His Leu
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Val Thr Leu Gly Gly Val Leu Arg Gln Gln Leu Val Val Ser Lys
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                                      25
Glu Leu Arg Met Tyr Asp Glu Arg Ala Gln Glu Trp Arg Ser Leu
                 35
                                      40
                                                          45
Ala Pro Met Asp Ala Pro Arg Tyr Gln His Gly Tyr Trp Leu Phe
                 50
                                      55
Ile Gly Asn Phe Leu Tyr Val Val Gly Gly Gln Ser Asn Tyr Asp
                                      70
                 65
Thr Lys Gly Lys Thr Ala Val Asp Thr Val Phe Arg Phe Asp Pro
                 80
                                      85
                                                          90
Arg Tyr Asn Lys Trp Met Gln Val Ala Ser Leu Asn Glu Lys Arg
                 95
                                     100
                                                          105
Thr Phe Phe His Leu Ser Ala Leu Lys Gly His Leu Tyr Ala Val
                110
                                     115
                                                         120
Gly Gly Arg Ser Ala Ala Gly Glu Leu Gly Thr Val Glu Cys Tyr
                125
                                     130
                                                          135
Asn Pro Arg Met Asn Glu Trp Ser Tyr Val Ala Lys Met Ser Glu
                140
                                                          150
                                     145
Pro His Tyr Gly His Ala Gly Thr Val Tyr Gly Gly Leu Met Tyr
                155
                                     160
                                                          165
Ile Ser Gly Gly Ile Thr His Asp Thr Phe Gln Asn Glu Leu Met
                170
                                     175
Cys Phe Asp Pro Asp Thr Asp Lys Trp Met Gln Lys Ala Pro Met
                185
                                     190
Thr Thr Val Arg Gly Leu His Cys Met Cys Thr Arg Trp Arg
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<210> 74
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<400> 74
Tyr Ser Arg Ile Leu Ile Leu Gln Met Phe Ile Leu Gly Ala Ile
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Ile Gln Ile Leu Pro Trp Val Met Ala Ser Gln Asn Ser Lys His
                 20
                                      25
His Pro Glu Leu Val Asp Leu Phe Ser Arg Ser Gly Ile Tyr Ile
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40
Lys Gln Val Val Leu Cys Lys Phe His Ser Val Phe Leu Ser Gln
Lys Gly Gln Val Tyr Thr Cys Gly His Gly Pro Gly Arg Ala Ile
                 65
                                      70
Arg Asp Met Gly Asp Glu Gln Thr Cys Leu Val Pro Arg Leu Val
                 80
                                      85
Glu Gly Leu Asn Gly His Asn Cys Ser Gln Val Ala Ala Ala Lys
                 95
                                     100
                                                          105
Asp His Thr Val Val Leu Thr Glu Asp Gly Cys Val Tyr Thr Phe
                110
                                     115
                                                          120
Gly Leu Asn Ile Phe His Gln Leu Gly Ile Ile Pro Pro Pro Ser
                125
                                     130
Ser Cys Asn Val Pro Arg Gln Ile Gln Ala Lys Tyr Leu Lys Gly
                140
                                     145
                                                          150
Arg Thr Ile Ile Gly Val Ala Ala Gly Arg Phe His Thr Val Leu
                155
                                     160
Trp Thr Arg Glu Ala Val Tyr Thr Met Gly Leu His Gly Gly Gln
                170
                                     175
Leu Gly Cys Leu Leu Asp Pro Asn Gly Glu Lys Cys Val Thr Ala
                185
                                     190
Pro Arg Gln Val Ser Ala Leu His His Lys Asp Ile Ala Leu Ser
                200
                                     205
                                                          210
Leu Val Ala Ala Ser Asp Gly Ala Thr Val Cys Val Thr Thr Arg
                215
                                     220
                                                          225
Gly Asp Ile Tyr Leu Leu Ala Asp Tyr Gln Cys Lys Lys Met Ala
                230
                                     235
Ser Lys Gln Leu Asn Leu Lys Lys Val Leu Val Ser Gly Gly His
                245
                                     250
                                                          255
Met Glu Tyr Lys Val Asp Pro Glu His Leu Lys Glu Asn Gly Gly
                260
                                     265
                                                          270
Gln Lys Ile Cys Ile Leu Ala Met Asp Gly Ala Gly Arg Val Phe
                275
                                     280
Cys Trp Arg Ser Val Asn Ser Ser Leu Lys Gln Cys Arg Leu Gly
                290
                                     295
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Leu Ser Thr Ser Gly Ser Ser Phe Leu Ile Trp Leu
                305
                                     310 .
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<400> 75
Leu Tyr Val Met Leu Glu Met Thr Arg Pro Ser Ser Leu Ser Leu
                                      10
Ser Gln Leu Ala Leu Phe Ser Arg Ala Val Leu Pro Val Gly Arg
                                      25
Ala Glu Asp Leu Ala Gly Glu Ala Gly Glu Ala Cys Trp Pro Ser
                 35
                                      40
                                                           45
Leu Cys Ala Pro Leu His Ala His Pro Pro Ala Pro Pro Glu Arg
                 50
Ile Val His Pro Ala Ala Arg Ser Leu Asp Leu His Phe Gly Ala
                 65
                                      70
Pro Gly Arg Val Glu Leu Arg Cys Glu Val Ala Pro Ala Gly Ser
                 80
                                      85
Gln Val Arg Trp Tyr Lys Asp Gly Leu Glu Val Glu Ala Ser Asp
                 95
                                     100
                                                          105
Ala Leu Gln Leu Gly Ala Glu Gly Pro Thr Arg Thr Leu Thr Leu
                110
                                     115
                                                          120
Pro His Ala Gln Pro Glu Asp Ala Gly Glu Tyr Val Cys Glu Thr
                125
                                     130
Arg His Glu Ala Ile Thr Phe Asn Val Ile Leu Ala Glu Pro Pro
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140
                                     145
Val Gln Phe Leu Ala Leu Glu Thr Thr Pro Ser Pro Leu Cys Val
                155
                                     160
                                                          165
Gly Pro Gly Glu Pro Val Val Gln Glu Gly Glu Gly Leu Glu Leu
                                     175
                170
His Ala Glu Gly Pro Ala Glu Ser Leu His
<210> 76
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Arg Thr Cys Cys Arg Val Val Pro Glu Ala Lys Gln Arg Trp Arg
Arg Val Arg Leu Arg Arg Arg Gln Arg Arg Ala Pro Gly Arg Arg
                 20
                                      25
                                                           30
Ala Pro Gly Arg Ala Ala Leu Leu Val Leu Leu Ala Leu Ala Ala
                 35
                                      40
Ala Ala Ala Gly Ser Gly Arg Leu Ser Cys Arg Met Cys Gly Arg
                                      55
                 50
                                                           60
Arg Arg Ser Val Gly Gly Ala Gly Gly Pro Gly Ser Gly Leu
                                      70
Ala Pro Leu Pro Gly Leu Pro Pro Ser Ala Ala Ala His Gly Ala
                 80
                                                           90
                                      85
Ala Leu Leu Ser His Trp Asp Pro Thr Leu Ser Ser Asp Trp Asp
                 95
                                     100
                                                          105
Gly Glu Arg Thr Ala Pro Gln Cys Leu Leu Arg Ile Lys Arg Asp
                110
                                     115
Ile Met Ser Ile Tyr Lys Glu Pro Pro Pro Gly Met Phe Val Val
                125
                                     130
                                                          135
Pro Asp Thr Val Asp Met Thr Lys Ile His Ala Leu Ile Thr Gly
                140
                                     145
                                                          150
Pro Phe Asp Thr Pro Tyr Glu Gly Gly Phe Phe Leu Phe Val Phe
                155
                                     160
                                                          165
Arg Cys Pro Pro Asp Tyr Pro Ile His Pro Pro Arg Val Lys Leu
                170
                                     175
                                                          180
Met Thr Thr Gly Asn Asn Thr Val Arg Phe Asn Pro Asn Phe Tyr
                185
                                     190
                                                          195
Arg Asn Gly Lys Val Cys Leu Ser Ile Leu Gly Thr Trp Thr Gly
                200
                                     205
                                                          210
Pro Ala Trp Ser Pro Ala Gln Ser Ile Ser Ser Val Leu Ile Ser
                215
                                     220
                                                          225
Ile Gln Ser Leu Met Thr Glu Asn Pro Tyr His Asn Glu Pro Gly
                230
                                     235
                                                          240
Phe Glu Gln Glu Arg His Pro Gly Asp Ser Lys Asn Tyr Asn Glu
                                     250
                245
                                                          255
Cys Ile Arg His Glu Thr Ile Arg Val Ala Val Cys Asp Met Met
                260
                                     265
                                                          270
Glu Gly Lys Cys Pro Cys Pro Glu Pro Leu Arg Gly Val Met Glu
                275
                                     280
                                                          285
Lys Ser Phe Leu Glu Tyr Tyr Asp Phe Tyr
                290
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<211> 288
<212> PRT
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<400> 77

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Ala Pro Arg Leu Trp Ala Cys Pro Cys His Cys Trp Trp Ser Gly
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Ser Gly Pro Pro Ala Arg Cys Pro Tyr Ile Ile Gln Lys Cys Val
                 20
                                      25
                                                          30
Gly Gln Ile Glu Arg Arg Gly Leu Arg Val Val Gly Leu Tyr Arg
                 35
                                      40
                                                          45
Leu Cys Gly Ser Ala Ala Val Lys Lys Glu Leu Arg Asp Ala Phe
                 50
                                      55
Glu Arg Asp Ser Ala Ala Val Cys Leu Ser Glu Asp Leu Tyr Pro
                 65
                                      70
Asp Ile Asn Val Ile Thr Gly Ile Leu Lys Asp Tyr Leu Arg Glu
                                      85
                 80
                                                          90
Leu Pro Thr Pro Leu Ile Thr Gln Pro Leu Tyr Lys Val Val Leu
                 95
                                     100
Glu Ala Met Ala Pro Gly Thr Pro Gln Thr Glu Phe Pro Pro Pro
                110
                                     115
                                                         120
Leu Arg Ala Pro Glu Gly Ser Tyr Ser Cys Leu Pro Asp Val Glu
                125
                                     130
                                                         135
Arg Ala Thr Leu Thr Leu Leu Leu Asp His Leu Arg Leu Val Ser
                140
                                     145
Ser Phe His Ala Tyr Asn Arg Met Thr Pro Gln Asn Leu Ala Val
                155
                                     160
                                                         165
Cys Phe Gly Pro Val Leu Leu Pro Ala Arg Gln Ala Pro Thr Arg
                170
                                     175
                                                          180
Pro Arg Ala Arg Ser Ser Gly Pro Gly Leu Ala Ser Ala Val Asp
                185
                                     190
                                                         195
Phe Lys His His Ile Glu Val Leu His Tyr Leu Leu Gln Ser Trp
                200
                                     205
                                                         210
Pro Asp Pro Arg Leu Pro Arg Gln Ser Pro Asp Val Ala Pro Tyr
                                     220
Leu Arg Pro Lys Arg Gln Pro Pro Leu His Leu Pro Leu Ala Asp
                230
                                     235
                                                         240
Pro Glu Val Val Thr Arg Pro Arg Gly Arg Gly Pro Glu Ser
                245
                                     250
                                                         255
Pro Pro Ser Asn Arg Tyr Ala Gly Asp Trp Ser Val Cys Gly Arg
                260
                                     265
                                                         270
Gly Leu Pro Asp Leu Trp Ala Gly Phe Pro Val Arg Ala Arg Leu
                275
                                     280
                                                         285
Arg Pro Leu
<210> 78
<211> 294
<212> PRT
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<400> 78
Leu Ala Ala Pro Gln Ser His Ser Ile Pro Ser Pro Pro Gly Ala
His Leu Leu Lys Thr Arg Val Leu Pro Ser Ala Arg Arg Ala Arg
                 20
                                      25
                                                           30
Ala Arg Gly Ala Arg Glu Leu Arg Ser Ala Arg Ala Met Gly Pro
Pro Pro Gly Ala Gly Val Ser Cys Arg Gly Gly Cys Gly Phe Ser
                                      55
Arg Leu Leu Ala Trp Cys Phe Leu Leu Ala Leu Ser Pro Gln Ala
                 65
                                      70
Pro Gly Ser Arg Gly Ala Glu Ala Val Trp Thr Ala Tyr Leu Asn
                 80
                                      85
                                                          90
Val Ser Trp Arg Val Pro His Thr Gly Val Asn Arg Thr Val Trp
                 95
                                                         105
                                     100
Glu Leu Ser Glu Glu Gly Val Tyr Gly Pro Asp Ser Pro Leu Glu
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110
                                     115
Pro Val Ala Gly Val Leu Val Pro Pro Asp Gly Pro Gly Ala Leu
                125
                                     130
                                                         135
Asn Ala Cys Asn Pro His Thr Asn Phe Thr Val Pro Thr Val Trp
                140
                                                         150
                                     145
Gly Ser Thr Val Gln Val Ser Trp Leu Gly Leu Ile Gln Arg Gly
                155
                                     160
                                                         165
Gly Gly Cys Thr Phe Ala Asp Lys Ile His Leu Ala Tyr Glu Arg
                170
                                     175
                                                         180
Gly Ala Ser Gly Ala Val Ile Phe Asn Phe Pro Gly Thr Arg Asn
                185
                                     190
                                                         195
Glu Val Ile Pro Met Ser His Pro Gly Ala Val Asp Ile Val Ala
                200
                                     205
                                                         210
Ile Met Ile Arg Gln Ser Glu Arg His Lys Asn Ser Ala Ile Tyr
                215
                                     220
                                                         225
Ser Lys Arg His Thr Ser Asp Asn Gly His Arg Ser Arg Glu Lys
                230
                                     235
                                                         240
Thr Trp Pro Leu Gly Glu Ser Leu Phe Asn Phe Phe Arg Phe Leu
                245
                                     250
                                                         255
Cys Pro Phe Leu Leu Arg Arg Ala Thr Val Gly Tyr Phe Ile
                260
                                     265
                                                         270
Phe Tyr Ser Ala Arg Arg Leu Arg Asn Ala Arg Ala Gln Ser Arg
                275
                                     280
Lys Gln Arg Pro Ile Lys Gly Arg Cys
                290
<210> 79
<211> 196
<212> PRT
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Gly Ala Thr Pro Arg Ala Gly Glu Arg Ala Pro Leu Leu Pro Asp
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Arg Ala Ala His Ala Ala Ser Gly Thr Ile Thr Val Ala Gly Arg
                 20
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Arg Pro Val Gln Ile Leu Ser Glu Phe Phe Gly Ala Phe Ser Pro
                                                           45
                                      40
Arg Lys Leu Ala Ile Gln Lys Cys Ala Ser Arg Thr Ala Ala Ala
                 50
                                      55
Met Gly Ser Glu Asp His Gly Ala Gln Lys Pro Ser Cys Lys Ile
                 65
                                      70
Met Thr Phe Arg Pro Thr Met Gly Glu Phe Lys Asp Phe Asn Lys
                 80
                                      85
Tyr Val Gly Tyr Ile Glu Ser Gln Gly Ala His Arg Ala Gly Leu
                 95
                                     100
                                                          105
Gly Lys Ile Ile Pro Pro Lys Glu Trp Lys Pro Arg Gln Thr
                                                         Tvr
                110
                                     115
                                                          120
Asp Asp Ile Asp Asp Val Val Ile Pro Gly Pro Ile Gln Gln Val
                125
                                     130
                                                          135
Val Thr Gly Gln Ser Gly Leu Phe Thr Gln Tyr Asn Ile Gln Lys
                140
                                     145
                                                          150
Lys Gly Met Thr Val Gly Glu Tyr Arg Arg Leu Gly Asn Ser Glu
                155
                                     160
Lys Tyr Cys Thr Pro Arg Asp Gln Asp Phe Asp Asp Leu Glu Arg
                170
                                     175
Lys Tyr Trp Glu Gly Thr Leu Thr Leu Cys Leu Pro Asp Leu Arg
                185
                                     190
                                                          195
Gly
<210> 80
<211> 745
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<212> PRT

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445
                440
Pro Asn Lys Glu Glu Glu Glu Gly Gly Gly Ala Arg Val Pro Ser
                                     460
Ala Pro Ala Pro Ser Leu Ala Tyr Gly Ala Pro Ala Ala Pro Leu
                470
                                     475
                                                         480
Ser Arg Pro Ala Ala Thr Met Val Thr Asn Val Val Arg Pro Val
                485
                                     490
                                                         495
Ser Ser Thr Pro Val Pro Ile Ala Ser Lys Pro Phe Pro Thr Ser
                500
                                     505
                                                         510
Gly Arg Ala Glu Ala Ser Pro Asn Asp Thr Ala Gly Ala Arg
                                                         Thr
                515
                                     520
                                                         525
Glu Met Gly Thr Gly Ser Arg Val Pro Gly Gly Ser Pro Leu Gly
                530
                                     535
                                                         540
Val Ser Leu Val Tyr Ser Asp Lys Lys Ser Ala Ala Ala Thr Ser
                545
                                     550
                                                         555
Pro Ala Pro His Leu Val Ala Gly Pro Leu Leu Gly Thr Val Gly
                                                         570
                560
                                     565
Lys Ala Pro Ala Thr Val Thr Asn Leu Leu Val Gly Thr Pro Gly
                575
                                     580
                                                         585
Tyr Gly Ala Pro Ala Pro Pro Ala Val Gln Phe Ile Ala Gln Gly
                590
                                     595
                                                          600
Ala Pro Gly Gly Gly Thr Thr Ala Gly Ser Gly Ala Gly Ala Gly
                605
                                     610
                                                         615
Ser Gly Pro Asn Gly Pro Val Pro Leu Gly Ile Leu Gln Pro Gly
                620
                                     625
                                                         630
Ala Leu Gly Lys Ala Gly Gly Ile Thr Gln Val Gln Tyr Ile Leu
                635
                                     640
Pro Thr Leu Pro Gln Gln Leu Gln Val Ala Pro Ala Pro Ala Pro
                650
                                     655
                                                         660
Ala Pro Gly Thr Lys Ala Ala Pro Met Arg Pro Cys Thr His
                665
                                     670
                                                          675
His Gln His Pro Phe His Pro Pro Thr Gly His Phe His Gln Arg
                680
                                     685
                                                          690
Gln Ser Pro Gly Cys His Cys Thr His Ser Trp His Pro His Pro
                695
                                     700
                                                          705
Ala Val Cys Thr Leu Arg Pro Thr Pro Gln Ser Pro Val Ser Phe
                710
                                     715
                                                          720
Ser Arg Ala Gly Pro Ala Pro Gly Trp Leu Ser Pro Ala Ala Ala
                725
                                     730
Trp Glu Gly Pro Ser Ala Ser Gly Arg Pro
                740
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<400> 81
Leu Ala Met Lys Asp Met Leu Thr Val Val Asp Leu Leu Glu
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Gly Gly Ala Asp Val Asp His Thr Asp Asn Asn Gly Arg Thr Pro
Leu Leu Ala Ala Ser Met Gly His Ala Ser Val Val Asn Thr
                 35
                                      40
Leu Leu Phe Trp Gly Ala Ala Val Asp Ser Ile Asp Ser Glu Gly
                 50
                                     55
Arg Thr Val Leu Ser Ile Ala Ser Ala Gln Gly Asn Val Glu Val
                 65
                                      70
Val Arg Thr Leu Leu Asp Arg Gly Leu Asp Glu Asn His Arg Asp
                 80
                                      85
                                                           90
Asp Ala Gly Trp Thr Pro Leu His Met Ala Ala Phe Glu Gly His
                 95 ·
                                     100
Arg Leu Ile Cys Glu Ala Leu Ile Glu Gln Gly Ala Arg Thr Asn
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110
                                     115
Glu Ile Asp Asn Asp Gly Arg Ile Pro Phe Ile Leu Ala Ser Gln
                125
Glu Gly His Tyr Asp Cys Val Gln Ile Leu Leu Glu Asn Lys Ser
                140
                                     145
                                                          150
Asn Ile Asp Gln Arg Gly Tyr Asp Gly Arg Asn Ala Leu Arg Val
                155
                                     160
Ala Ala Leu Glu Gly His Arg Asp Ile Val Glu Leu Leu Phe Ser
                170
                                     175
                                                          1.80
His Gly Ala Asp Val Asn Cys Lys Asp Ala Asp Gly Arg Pro Thr
                185
                                     190
                                                          195
Leu Tyr Ile Leu Ala Leu Glu Asn Gln Leu Thr Met Ala Glu Tyr
                200
                                     205
                                                          210
Phe Leu Glu Asn Gly Ala Asn Val Glu Ala Ser Asp Ala Glu Gly
                215
                                                          225
                                     220
Arg Thr Ala Leu His Val Ser Cys Trp Gln Gly His Met Gly Asn
                230
                                     235
Gly Ala Gly Pro Asp Ser Ile Pro Cys Arg Arg Gln Cys Cys Arg
                245
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Gln
<210> 82
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<400> 82
Met Pro Ile Leu Pro Ile Ser Val Gln Leu Asp Ala Ser Leu Leu
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Ile Cys Leu Val Ile Cys Ala Gly Arg Phe Trp Thr Asn Leu Tyr
                  20
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Ser Leu Thr Val Pro Phe Gly Gln Lys Pro Asn Ile Asp Val Thr
                 35
                                      40
                                                           45
Asp Ala Met Val Asp Gln Ala Trp Asp Ala Gln Arg Ile Phe Lys
                 50
                                      55
                                                           60
Glu Ser Ala Glu Leu Leu Cys Ile Cys Trp Ser Ser Leu Tyr Asp
Ser Arg Ile Leu Arg Gln Ile Pro Cys Tyr
                                         Thr Asp Pro Gly Asn
                 80
                                      85
Val Gln Lys Ala Leu Cys His Pro His Ser Leu Gly Pro Gly Glu
                 95
                                     100
Gly Arg Leu Gln Arg Ser Leu Cys Ala Gln Arg Val Thr Met Asp
                110
                                     115
                                                          120
Asp Phe Leu Thr Ala His His Glu Met Gly His Ile Gln Tyr Asp
                125
                                     130
Met Ala Tyr Ala Gly Gln Pro Phe Ser Ala Lys Glu Met Glu Leu
                140
                                     145
                                                          150
Asn Glu Gly Phe His Glu Ala Val Gly Glu Ile Met Ser Leu Ser
                155
                                     160
                                                          165
Ala Ala Thr Pro Lys His Leu Lys Ser Ile Gly Leu Leu Ser Pro
                170
                                     175
                                                          180
Glu Phe Ser Thr Asn Asp Asn Glu Thr Glu Ile Asn Phe Leu Leu
                185
                                     190
                                                          195
Lys Gln Ala Leu Thr Ile Val Gly Thr Leu Pro Phe Thr Tyr Met
                200
                                     205
                                                          210
Leu Glu Lys Trp Arg Trp Met Val Phe Lys Arg Gly Asn Ser Gln
                215
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Arg Pro Val Gly Glu Lys Gly Gly Arg
                230
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<210> 83
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Asn Met Ala Gln Phe Tyr Tyr Lys Arg Asn Val Asn Ala Pro Tyr
                                      10
Arg Asp Arg Ile Pro Leu Arg Ile Val Arg Ala Glu Ser Glu Leu
                                      25
                 20
Ser Pro Ser Glu Lys Ala Tyr Leu Asn Ala Val Glu Lys Gly Asp
                                      40
                                                           45
Tyr Ala Ser Val Lys Lys Ser Leu Glu Glu Ala Glu Ile Tyr Phe
                 50
                                      55
                                                           60
Lys Ile Asn Ile Asn Cys Ile Asp Pro Leu Gly Arg Thr Ala Leu
Leu Ile Ala Ile Glu Asn Glu Asn Leu Glu Leu Ile Glu Leu Leu
                 80
                                      85
Leu Ser Phe Asn Val Tyr Val Gly Asp Ala Leu Leu His Ala Ile
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Arg Lys Glu Val Val Gly Ala Val Glu Leu Leu Leu Asn His Lys
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Lys Pro Ser Gly Glu Lys Gln Val Pro Pro Ile Leu Leu Asp Lys
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Gln Phe Ser Glu Phe Thr Pro Asp Ile Thr Pro Ile Ile Leu Ala
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Ala His Thr Asn Asn Tyr Glu Ile Ile Lys Leu Leu Val Gln Lys
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Gly Val Ser Val Pro Arg Pro His Glu Val Arg Cys Asn Cys Val
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Glu Cys Val Ser Ser Ser Asp Val Asp Ser Leu Arg His Ser Arg
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Ser Arg Leu Asn Ile Tyr Lys Ala Leu Ala Ser Pro Ser Leu Ile
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Ala Leu Ser Ser Glu Asp Pro Phe Leu Thr Ala Phe Gln Leu Ser
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Trp Glu Leu Gln Glu Leu Ser Lys Val Glu Asn Glu Phe Lys Ser
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Glu Tyr Glu Glu Leu Ser Arg Gln Cys Lys Gln Phe Ala Lys Asp
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Leu Leu Asp Gln Thr Arg Ser Ser Arg Glu Leu Glu Ile Ile Leu
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Asn Tyr Arg Asp Asp Asn Ser Leu Ile Glu Glu Gln Ser Gly Asn
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Asp Leu Ala Arg Leu Lys Leu Ala Ile Lys Tyr Arg Gln Lys Glu
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Phe Val Ala Gln Pro Asn Cys Gln Gln Leu Leu Ala Ser Arg Trp
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Tyr Asp Glu Phe Pro Gly Trp Arg Arg Arg His Trp Ala Val Lys
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Met Val Thr Cys Phe Ile Ile Gly Leu Leu Phe Pro Val Phe Ser
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                                     340
Val Cys Tyr Leu Ile Ala Pro Lys Ser Pro Leu Gly Leu Phe Ile
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Arg Lys Pro Phe Ile Lys Phe Ile Cys His Thr Ala Ser Tyr Leu
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Thr Phe Leu Phe Leu Leu Leu Ala Ser Gln His Ile Asp Arg
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Ser Asp Leu Asn Arg Gln Gly Pro Pro Pro Thr Ile Val Glu Trp
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Met Ile Leu Pro Trp Val Leu Gly Phe Ile Trp Gly Glu Ile Lys
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Gln Met Trp Asp Gly Gly Leu Gln Asp Tyr Ile His Asp Trp Trp
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Asn Leu Met Asp Phe Val Met Asn Ser Leu Tyr Leu Ala Thr Ile
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Ser Leu Lys Ile Val Ala Phe Val Lys Tyr Ser Ala Leu Asn Pro
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Arg Glu Ser Trp Asp Met Trp His Pro Thr Leu Val Ala Glu Ala
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Leu Phe Thr Ala Asn Ser His Leu Gly Pro Leu Gln Ile Ser Leu
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Gly Arg Met Leu Leu Asp Ile Leu Lys Phe Leu Phe Ile Tyr Cys
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Leu Val Leu Leu Ala Phe Ala Asn Gly Leu Asn Gln Leu Tyr Phe
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Tyr Tyr Glu Glu Thr Lys Gly Leu Thr Cys Lys Gly Ile Arg Cys
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Glu Lys Gln Asn Asn Ala Phe Ser Thr Leu Phe Glu Thr Leu Gln
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Ser Leu Phe Trp Ser Ile Phe Gly Leu Ile Asn Leu Tyr Val Thr
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Asn Val Lys Ala Gln His Glu Phe Thr Glu Phe Val Gly Ala Thr
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Thr Cys
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Ser Tyr Val Pro Val Ser Ala Pro Pro Pro Asn Ser Ser Glu Gln
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Tyr Ser Ser Gly Ala Gln Ser Ile Pro Ser Thr Val Thr Val Ile
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Ala Pro Trp Ser Pro Thr Leu Glu Asn Thr Trp Glu Leu Val
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                                      70
Leu Leu Leu Lys Ile Ile Ser Ser Ser Asn Ser Phe Gly Arg
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Asn Leu Pro Pro Lys Arg Arg Cys Arg Asp Tyr Asp Glu Arg Gly
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Phe Cys Val Leu Gly Asp Leu Cys Gln Phe Asp His Gly Asn Asp
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Pro Leu Val Val Asp Glu Val Ala Leu Pro Ser Met Ile Pro Phe
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Pro Pro Pro Pro Pro Gly Leu Pro Pro Pro Thr Thr Pro Gly Met
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Leu Met Pro Pro Met Pro Gly Pro Gly Pro Gly Pro Gly
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Pro Gly Pro Gly Pro Gly Pro Gly Pro Gly His Ser Met
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Arg Leu Pro Val Pro Gln Gly His Gly Gln Pro Pro Pro Ser Val
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Val Leu Pro Ile Pro Arg Pro Pro Ile Thr Gln Ser Ser Leu Ile
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                                     205
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Asn Ser Arg Asp Gln Pro Gly Thr Ser Ala Val Pro Asn Leu Ala
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                                     220
Ser Val Gly Thr Arg Leu Pro Pro Pro Leu Pro Gln Asn Leu Leu
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240
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Tyr Thr Val Ser Glu Arg Gln Pro Met Tyr Ser Arg Glu His Gly
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Ala Ala Ala Ser Glu Arg Leu Gln Leu Gly Thr Pro Pro Pro
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Leu Ala Ala Arg Leu Val Pro Pro Arg Asn Leu Met Gly Ser Ser
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Ile Gly Tyr His Thr Ser Val Ser
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Glu Leu Phe His Pro Thr Leu Ala Ser Ile Ser Ser Pro Met Leu
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Glu Gly Ala Glu Leu Tyr Phe Asn Val Asp His Gly Tyr Leu Glu
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Gly Leu Val Arg Gly Cys Lys Ala Ser Leu Leu Thr Gln Gln Asp
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                                      70
Tyr Ile Asn Leu Val Gln Cys Glu Thr Leu Glu Ala Pro Phe Phe
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Gln Asp Cys Met Ser Glu Asn Ala Leu Asp Glu Leu Asn Ile Glu
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Leu Leu Arg Asn Lys Leu Tyr Lys Ser Tyr Leu Glu Ala Phe Tyr
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                                     115
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Lys Phe Cys Lys Asn His Gly Asp Val Thr Ala Glu Val Met Cys
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Pro Ile Leu Glu Phe Glu Ala Asp Arg Arg Ala Phe Ile Ile Thr
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Leu Asn Ser Phe Gly Thr Glu Leu Ser Lys Glu Asp Arg Glu Thr
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Leu Tyr Pro Thr Phe Arg Gln Leu Tyr Pro Glu Gly Leu Arg Leu
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                                     175
Leu Ala Gln Ala Glu Asp Phe Asp Gln Met Lys Asn Val Ala Asp
                185
                                     190
His Tyr Gly Val Tyr Lys Pro Leu Phe Glu Ala Val Gly Gly Ser
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Gly Gly Lys Thr Leu Glu Asp Val Phe Tyr Glu Arg Glu Val Gln
                215
                                     220
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Met Asn Val Leu Ala Phe Asn Arg Gln Phe His Tyr Gly Val Phe
                230
                                     235
                                                          240
Tyr Ala Tyr Val Lys Leu Lys Glu Gln Glu Ile Arg Asn Ile Val
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Trp Ile Ala Glu Cys Ile Ser Gln Arg His Arg Thr Lys Ile Asn
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Ser Tyr Ile Pro Ile Leu
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Gln Thr Ser Tyr Thr Leu Phe Val Arg Glu Asn Asn Ser Pro Ala
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Leu His Ile Gly Ser Val Ser Ala Thr Asp Arg Asp Ser Gly Thr
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Asn Ala Gln Val Thr Tyr Ser Leu Leu Pro Pro Gln Asp Pro His
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                                      85
Leu Pro Leu Ala Ser Leu Val Ser Ile Asn Ala Asp Asn Gly His
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Leu Phe Ala Leu Arg Ser Leu Asp Tyr Glu Ala Leu Gln Ala Phe
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Glu Phe Arg Val Gly Ala Ser Asp Arg Gly Ser Pro Ala Leu Ser
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Ser Glu Ala Leu Val Arg Val Leu Val Leu Asp Thr Asn Asp Asn
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                                     145
Ser Pro Phe Val Leu Tyr Pro Leu Gln Asn Gly Ser Ala Pro Cys
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                                     160
                                                         165
Thr Glu Leu Val Pro Arg Ala Ala Glu Pro Gly Tyr Leu Val Thr
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                                     175
                                                         180
Lys Val Val Ala Val Asp Gly Asp Ser Gly Gln Asn Ala Trp Leu
                185
                                     190
Ser Tyr Gln Leu Leu Lys Ala Thr Glu Pro Gly Leu Phe Gly Val
                200
                                     205
                                                         210
Trp Ala His Asn Gly Glu Val Arg Thr Ala Arg Leu Leu Ser Glu
                215
                                     220
                                                         225
Arg Asp Ala Ala Lys His Arg Leu Val Val Leu Val Lys Asp Asn
                230
                                     235
Gly Glu Pro Pro Arg Ser Ala Thr Ala Thr Leu His Val Leu Leu
                245
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                                                         255
Val Asp Gly Phe Ser Gln Pro Tyr Leu Pro Leu Pro Glu Ala Ala
                260
                                     265
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Pro Ala Gln Ala Gln Ala Asp Ser Leu Thr Val Tyr Leu Val Val
                275
                                     280
Ala Leu Ala Ser Val Ser Ser Leu Phe Leu Phe Ser Val Leu Leu
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                                     295
                                                         300
Phe Val Ala Val Arg Leu Cys Arg Arg Ser Arg Ala Ala Ser Val
                305
                                     310
Gly Arg Cys Ser Val Pro Glu Gly Pro Phe Pro Gly His Leu Val
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                                     325
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Asp Val Ser Gly Thr Gly Thr Leu Ser Gln Glu Leu Pro Val Arg
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Gly Val Ser Asp Arg Arg Leu Trp Asp Trp
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Ser Gly Gln Leu His Tyr Ser Val Pro Glu Glu Ala Glu His Gly
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Thr Phe Val Gly Arg Ile Ala Gln Asp Leu Gly Leu Glu Leu Ala
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Glu	Leu	Val	Pro	Arg 65	Leu	Phe	Gln	Leu	Asp 70	ser	Lys	GJÀ	Arg	Gly 75
Asp	Leu	Leu	Glu	Val 80	Asn	Leu	Gln	Asn	Gly 85	Ile	Leu	Phe	Val	Asn 90
Ser	Arg	Ile	Asp	Arg 95	Glu	Glu	Leu	Cys	Gly 100	Arg	Ser	Ala	Glu	Cys 105
Ser	Ile	His	Leu	Glu 110	Val	Ile	Val	Asp	Arg 115	Pro	Leu	Gln	Val	Phe 120
His	Val	Asp	Val	Glu 125	Val	Lys	Asp	Ile	Asn 130	Asp	Asn	Pro	Pro	Val 135
Phe	Pro	Ala	Thr	Gln 140	Lys	Asn	Leu	Phe	Ile 145	Ala	Glu	Ser	Arg	Pro 150
Leu	Asp	Ser	Arg	Phe 155	Pro	Leu	Glu	Gly	Ala 160	Ser	Asp	Ala	Asp	Ile 165
Gly	Glu	Asn	Ala	Leu 170	Leu	Thr	Tyr	Arg		Ser	Pro	Asn	Glu	Tyr 180
Phe	Phe	Leu	Asp	Val 185	Pro	Thr	Ser	Asn	Gln 190	Gln	Val	Lys	Pro	Leu 195
Gly	Leu	Val	Leu	Arg 200	Lys	Leu	Leu	Asp	Arg 205	Glu	Glu	Thr	Pro	Glu 210
Leu	His	Leu	Leu	Leu 215	Thr	Ala	Thr	Asp	Gly 220	Gly	Lys	Pro	Glu	Leu 225
Thr	Gly	Thr	Val	Gln 230	Leu	Leu	Ile	Thr	Val 235	Leu	Asp	Asn	Asn	Asp 240
Asn	Ala	Pro	Val	Phe 245	Asp	Arg	Thr	Leu	Tyr 250	Thr	Val	Lys	Leu	Pro 255
Glu	Asn	Val	Ser	Ile 260	Gly	Thr	Leu	\Va1	Ile 265	His	Pro	Asn	Ala	Ser 270
Asp	Leu	Asp	Glu	Gly 275	Leu	Asn	Gly	Asp	Ile 280	Ile	Tyr	Ser	Phe	Ser 285
Ser	Asp	Val	Ser	Pro 290	Asp	Ile	Lys	Ser	Lys 295	Phe	His	Met	Asp	Pro 300
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ser	Arg	Ala	His	<b>Lys</b> 320	Ile	Pro	Val	Glu	Ala 325	Val	Asp	Lys	Gly	Phe 330
Pro	Pro	Leu	Ala	Gly 335	His	Суѕ	Thr	Leu	Leu 340	Val	Glu	Val	Val	Asp 345
Val	Asn	Asp	Asn	Ala 350	Pro	Gln	Leu	Thr	Ile 355	Lys	Thr	Leu	Ser	Val 360
Pro	Val	Lys	Glu	Asp 365	Ala	Gln	Leu	Gly	Thr 370	Val	Ile	Ala	Leu	Ile 375
Ser	Val	Ile	Asp	Leu 380	Asp	Ala	Asp	Ala	Asn 385	Gly	Gln	Val	Thr	Cys 390
Ser	Leu	Thr	Pro	His 395	Val	Pro	Phe	Lys	Leu 400		Ser	Thr	Tyr	Lys 405
Asn	Tyr	Tyr	Ser	Leu 410	Val	Leu	Asp	Arg	Ala 415	Leu	Asp	Arg	Glu	Ser 420
Val	Ser	Ala	Tyr	Glu 425	Leu	Val	Val	Thr	Ala 430	Arg	Asp	Gly	Gly	
Pro	Ser	Leu	Trp	Ala 440	Thr	Ala	Arg	Val	Ser 445	Val	Glu	Val	Ala	Asp 450
Val	Asn	Asp	Asn	Ala 455	Pro	Ala	Phe	Ala	Gln 460	Ser	Glu	Tyr	Thr	Val 465
Phe	Val	Lys	Glu	Asn 470	Asn	Pro	Pro	Gly	Cys 475	His	Ile	Phe	Thr	Val 480
Ser	Ala	Arg	Asp	Ala 485	Asp	Ala	Gln	Glu	Asn 490	Ala	Leu	Val	Ser	Tyr 495
Ser	Leu	Val	Glu	Arg 500	Arg	Leu	Gly	Glu	Arg 505	Ser	Leu	Ser	Ser	Tyr 510
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Leu	Asp	His	Glu	Glu 530	Leu	Glu	Leu	Leu		Phe	Gln	Val	Ser	
Arg	Asp	Ala	Gly	Val 545	Pro	Pro	Leu	Gly		Asn	Val	Thr	Leu	

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Val Phe Val Leu Asp Glu Asn Asp Asn Ala Pro Ala Leu Leu Thr
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Pro Arg Met Arg Gly Thr Asp Gly Ala Val Ser Glu Met Val Leu
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Arg Ser Val Gly Ala Gly Val Val Gly Lys Val Arg Ala Val
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Asp Ala Asp Ser Gly Tyr Asn Ala Trp Leu Ser Tyr Glu Leu Gln
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Pro Glu Thr Ala Ser Ala Ser Ile Pro Phe Arg Val Gly Leu Tyr
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Thr Gly Glu Ile Ser Thr Thr Arg Ala Leu Asp Glu Thr Asp Ala
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Pro Arg Gln Arg Leu Leu Val Leu Val Lys Asp His Gly Glu Pro
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Ala Leu Thr Ala Thr Ala Thr Val Leu Val Ser Leu Val Glu Ser
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Gly Gln Ala Pro Lys Ser Ser Ser Arg Ala Ser Val Gly Ala Thr
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                                     685
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Gly Pro Glu Val Thr Leu Val Asp Val Asn Val Tyr Leu Ile Ile
                695
                                    700
Ala Ile Cys Ala Val Ser Ser Leu Leu Val Leu Thr Leu Leu Leu
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Tyr Thr Val Leu Arg Cys Ser Ala Met Pro Thr Glu Gly Glu Cys
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Arg Ile Gln Phe Ser Asp Gly Asn Glu Phe Ala Val Asp Lys Ser
Lys Arg Gly Leu Ile His Val Pro Lys Asp Leu Pro Leu Lys Thr
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Lys Val Leu Asp Met Ser Gln Asn Tyr Ile Ala Glu Leu Gln Val
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Ser Asp Met Ser Phe Leu Ser Glu Leu Thr Val Leu Arg Leu Ser
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His Asn Arg Ile Gln Leu Leu Asp Leu Ser Val Phe Lys Phe Asn
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                                     100
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Gln Asp Leu Glu Tyr Leu Asp Leu Ser His Asn Gln Leu Gln Lys
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Ile Ser Cys His Pro Ile Val Ser Phe Arg His Leu Asp Leu Ser
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Phe Asn Asp Phe Lys Ala Leu Pro Ile Cys Lys Glu Phe Gly Asn
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Leu Ser Gln Leu Asn Phe Leu Gly Leu Ser Ala Met Lys Leu Gln
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Lys Leu Asp Leu Pro Ile Ala His Leu His Leu Ser Tyr Ile
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Leu Leu Asp Leu Arg Asn Tyr Tyr Ile Lys Glu Asn Glu Thr Glu
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Ser Leu Gln Ile Leu Asn Ala Lys Thr Leu His Leu Val Phe His
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Pro Thr Ser Leu Phe Ala Ile Gln Val Asn Ile Ser Val Asn Thr
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Cys	Gln	Val	Phe		Lys	Phe	Leu	ser		Leu	Thr	Arg	Gly	_
Thr	Leu	Leu	Asn		Thr	Leu	Asn	His		G1u	Thr	Thr	Trp	
Cys	Leu	Val	Arg		Phe	Gln	Phe	Leu		Pro	Lys	Pro	Val	
Tyr	Leu	Asn	Ile		Asn	Leu	Thr	Ile		Glu	Ser	Ile	Arg	
Glu	Asp	Phe	Thr	Tyr 305	Ser	Lys	Thr	Thr	Leu 310	Lys	Ala	Leu	Thr	Ile 315
Glu	His	Ile	Thr	Asn 320	Gln	Val	Phe	Leu	Phe 325	Ser	Gln	Thr	Ala	Leu 330
Tyr	Thr	Val	Phe	Ser	Glu	Met	Asn	Ile	Met 340	Met	Leu	Thr	Ile	Ser 345
Asp	Thr	Pro	Phe	Ile 350	His	Met	Leu	Cys	Pro 355	His	Ala	Pro	Ser	Thr 360
Phe	Lys	Phe	Leu	Asn 365	Phe	Thr	Gln	Asn	Val 370	Phe	Thr	Asp	Ser	Ile 375
Phe	Glu	Lys	Суѕ	Ser 380	Thr	Leu	Val	Lys	Leu 385	Glu	Thr	Leu	Ile	Leu 390
Gln	Lys	Asn	Gly	Leu 395	Lys	Asp	Leu	Phe	Lys 400	Val	Gly	Leu	Met	Thr 405
Lys	Asp	Met	Pro	Ser 410	Leu	Glu	Ile	Leu	Asp 415	Val	Ser	Trp	Asn	Ser 420
Leu	Glu	Ser	Gly	Arg 425	His	ГЛЗ	Glu	Asn	Cys 430	Thr	Trp	Val	Glu	Ser 435
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Phe	Arg	Суѕ	Leu	Pro 455	Pro	Arg	Ile	ГЛЗ	Val 460	Leu	Asp	Leu	His	Ser 465
Asn	ГЛЗ	Ile	ГЛЗ	Ser 470	Val	Pro	Lys	Gln	Val 475	Val	ГЛS	Leu	Glu	Ala 480
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Gly	Cys	Gly	Ser	Phe 500	Ser	Ser	Leu	Ser	Val 505	Leu	Ile	Ile	Asp	His 510
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				635					640			Gln	_	645
				650					655			Asp		660
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				680					685		_	Lys		690
				695		-			700		_	Lys		Ile 705
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Asn Leu Ile Leu Ile Leu Glu Pro Ile Pro Gln Asn Ser Ile
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Pro Asn Lys Tyr His Lys Leu Lys Ala Leu Met Thr Gln Arg Thr
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Thr Ser Pro Thr Ser Ser His Pro Pro Met Leu Pro His Pro Ser
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Thr Gly Ala Thr Asn Thr Leu Thr Gly Ser Ile Thr Arg Leu Leu
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His Lys Phe Thr Val Ile Ser Val Pro His Leu Pro Glu Lys Gln
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Ala Thr Gly Arg Phe Glu Glu Asp Phe Ile Glu Lys Arg Lys Arg
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Arg Leu Ile Leu Trp Met Asp His Met Thr Ser His Pro Val Leu
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Ser Gln Tyr Glu Gly Phe Gln His Phe Leu Ser Cys Leu Asp Asp
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Lys Gln Trp Lys Met Gly Lys Arg Arg Ala Glu Lys Asp Glu Met
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Val Gly Ala Ser Phe Leu Leu Thr Phe Gln Ile Pro Thr Glu His
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Gln Asp Leu Gln Asp Val Glu Asp Arg Val Asp Thr Phe Lys Ala
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Phe Ser Lys Lys Met Asp Asp Ser Val Leu Gln Leu Ser Thr Val
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Ala Ser Glu Leu Val Arg Lys His Val Gly Gly Phe Pro Gln Gly
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Ile Pro Glu Arg Trp Ala Val Pro Ser Arg Pro Ser Val Ile Pro
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Ser Arg Trp Thr Pro Pro Phe Ala Leu Arg Pro Ser Thr Val Pro
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Phe Leu Thr Arg Ala Val Pro Met Lys Pro Ser Gly Arg Cys Leu
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Leu Ser Ser Pro Arg Met Thr Ser Ser Arg Cys Trp Thr His Cys
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Leu Ser Thr Arg Ala Cys Ser Pro Thr Ser Leu Thr Ser Ser Ile
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Tyr Lys Lys Ala Pro Ser Pro Arg
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